THE DEVELOPMENT OF THE DIENCEPHALON OF THE CHINESE HAMSTER

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THE DEVELOPMENT OF THE DIENCEPHALON OF THE CHINESE HAMSTER

AN INVESTIGATION OF THE VALIDITY OF THE CRITERIA OF SUBDIVISION OF THE BRAIN

PROEFSCHRIFT

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AC	aqueductus cerebri (Sylvii)
CA	commissura anterior
CC	crus cerebri
CGLD	corpus geniculatum laterale pars dorsalis
CGLV	corpus geniculatum laterale pars ventralis
CGMD	corpus geniculatum mediale pars dorsalis
CGMV	corpus geniculatum mediale pars ventralis
CHO	chiasma opticum
CI	capsula interna
COP	commissura posterior
CP	caudatus putamen complex
CSL	corpus subthalamicum Luysi
D	nucleus of Darkschewitsch
EP	nucleus entopeduncularis
F	fornix
FM	foramen Monroi
FMT	fasciculus mamillotegmentalis
FR	fasciculus retroflexus
GP	globus pallidus
H_1	field H_1 of Forel
H_2	field H_2 of Forel
HA	nucleus anterior hypothalami
HCC	hypothalamic cell cord
HDM	nucleus dorsomedialis hypothalami
HI	nucleus infundibularis hypothalami
HL	nucleus lateralis hypothalami
HP	nucleus posterior hypothalami
HPV	nucleus paraventricularis hypothalami
HTL	nucleus tuberis lateralis hypothalami
HVM	nucleus ventromedialis hypothalami
I	nucleus interstitialis (of Cajal)
LH	nucleus habenularis lateralis
LM	lemniscus medialis
MFB	medial forebrain bundle
MH	nucleus habenularis medialis
ML	nucleus mamillaris lateralis
MM	nucleus mamillaris medialis
MMN	nucleus mamillaris medianus
MT	tractus mamillothalamicus
OC	nucleus oculomotorius
P	nucleus pretectalis
PD	nucleus premamillaris dorsalis
PDI	pronucleus of the nuclei of Cajal and Darkschewitsch
PF	nucleus parafascicularis
PO	nucleus posterior (thalami)
POL	nucleus preopticus lateralis
POM	nucleus preopticus medialis
POMA	nucleus preopticus magnocellularis
POP	nucleus preopticus periventricularis
PV	nucleus premamillaris ventralis
R	nucleus ruber
RE	nucleus reuniens
RH	nucleus rhomboideus
RM	recessus mamillaris
SC	nucleus suprachiasmaticus
SCO	organon subcommissurale
SGC	substantia grisea centralis
SI	substantia innominata
SM	stria medullaris
SMD	decussatio supramamillaris
SNC	substantia nigra pars compacta
SNR	substantia nigra pars reticulata
SO	nucleus supraopticus
SPC p g	suprapeduncular complex pars geniculata
SPC p l	suprapeduncular complex pars incerta
ST	stria terminalis
STN	bed nucleus of the stria terminalis
T	nucleus taeniae
TAD	nucleus anterior dorsalis thalami
TAM	nucleus anterior medialis thalami
TAV	nucleus anterior ventralis thalami
TL	nucleus lateralis thalami
TLP	nucleus lateralis posterior thalami
TMM	nucleus medialis thalami pars medialis
TML	nucleus medialis thalami pars lateralis
TO	tractus opticus
TOL	nucleus of the tractus olfactorius lateralis
TPA	nucleus paraventricularis thalami
TPT	nucleus parataenialis thalami
TR	nucleus reticularis thalami
TV	nucleus ventralis thalami
TVD	nucleus ventralis thalami pars dorsomedialis
TVM	nucleus ventralis thalami pars medialis
ZI	zona incerta
III	ventriculus tertius
IV	ventriculus quartus

arch.	archencephalon
b.l.	basilarleiste
c. call.	corpus callosum
cereb.	cerebellum
com. ant.	commissura anterior
com. hab.	commissura habenularum
com. hip.	commissura hippocampi
com. post.	commissura posterior
corp. mam.	corpus mamillare
cr. interpar.	crista interparencephalica
deut.	deuterencephalon
dienc.	diencephalon
em. thal.	eminentia thalami
ep.	epiphysis
epith.	epithalamus
f.l.m.	fasciculus longitudinalis medialis
hyp.	anlage of the adenohypophysis
hypoth.	hypothalamus
inf.	infundibulum
l. term.	lamina terminalis
mes.	mesencephalon
m.g.h.	medial ganglionic hill
m. interm.	massa intermedia
opt. st.	optic stalk
org. subforn.	organon subfornicale
par. ant.	parencephalon anterius
par. post.	parencephalon posterius
p. dors. thal.	pars dorsalis thalami
pl. ch.	plexus chorioideus
pl. enc. d.	plica encephali dorsalis
pl. enc. v.	plica encephali ventralis
preop.	regio preoptica
prerubr. tegm.	prerubral tegmentum
pret. p. precom.	pretectum pars precommissuralis
pros.	prosencephalon
pros. prim.	prosencephalon primitivum
pros. sec.	prosencephalon secundarium
p. ventr. thal.	pars ventralis thalami
rec. inframam.	recessus inframamillaris
rec. mam.	recessus mamillaris
rec. postopt.	recessus postopticus
rec. preopt.	recessus preopticus
rec. syn.	recessus synencephali
rec. supramam.	recessus supramamillaris
reg. mam.	regio mamillaris
reg. postopt.	regio postoptica
reg. preopt.	regio preoptica
reg. pret.	regio pretectalis
reg. subthal.	regio subthalamica
reg. supraopt.	regio supraoptica
rhomb.	rhombencephalon
s.d.b.	sulcus diencephalicus basalis
s.d.d.	sulcus diencephalicus dorsalis
s.d.m.	sulcus diencephalicus medius
s.d.v.	sulcus diencephalicus ventralis
s.i.a.	sulcus intraencephalicus anterior
s.h.	sulcus hemisphaericus
s. hyp. comp.	suprahypothalamic complex
s. lat. mes.	sulcus lateralis mesencephali
s.l.i.	sulcus lateralis infundibuli
s.lim.	sulcus limitans
str. med.	stria medullaris
str. term.	stria terminalis
subth. c.c.	subthalamic cell cord
syn.	synencephalon
tect. mes.	tectum mesencephali
tegm. c.c.	tegmental cell cord
tegm. mes.	tegmentum mesencephali
tel.	telencephalon
thal.	thalamus
tor. hem.	torus hemisphaericus
tor. tr.	torus transversus
tr. op.	tractus opticus
tr. zon. lim.	tract of the zona limitans intra thalamica
tub. mam.	tuberculum mamillare
tub. post.	tuberculum posterius
vel. tr.	velum transversum

Roman numerals were employed in order to indicate neuromeres in a number of the figures;

I	Telencephalic neuromere
II	Optic neuromere
III	Postoptic neuromere
IV	Parencephalon anterius
V	Parencephalon posterius
VI	Synencephalon
VII	Rostral mesencephalic neuromere
VIII	Caudal mesencephalic neuromere

CHAPTER I

INTRODUCTION

The division of the brain corresponding to the present concept of the diencephalon formerly was called thalamus opticus (Walker, '38; Meyer, '71)[1] and was believed to be the bed of the optic tract. His (1893[a]), on the basis of embryological work, proposed a subdivison of this part of the brain into three regions; he discerned an epithalamus, thalamus and hypothalamus which were arranged as longitudinal zones in superposition to one another. In addition he distinguished a metathalamus which corresponds to the corpora geniculata, and which forms a caudal extension of the thalamus. In 1895 this nomenclature was accepted by the Anatomische Gesellschaft, and it was incorporated in the "Baseler Nomina Anatomica" (His, 1895).

Comparative analysis of the morphology of the vertebrate brain in subsequent years, however, brought to light the insufficiency of this subdivision. A more extensive embryological background was needed in order to furnish detailed information on the basic plan underlying the complex structure of the adult brain. In comparative anatomy, the basic question is which parts of the brain are morphologically equivalent, *i.e.*, the establishment of homologies. In addition the homology has considerable practical significance; only entities which are in fact homologous deserve the same name in different species.

Without going into a discussion of the concept of homology[2] we will at once put forth the working definition of 'homology' that was used by Nieuwenhuys and Bodenheimer ('66): "Entities of different species which, within an obviously similar structural plan common to all species compared, occupy a corresponding topological position, should be considered homologous". This definition is in accord with the ideas of many comparative neurologists.

1. Probably, the word θαλαμη (G lurking place or lair) was used by Galen to indicate part of the lateral ventricle of the brain (Simon, '06). A confusion of this word with θαλαμος (G an inner room or bride-chamber) and a misinterpretation of Galen's description as concerning the third ventricle resulted in the use of the word thalamus for the indication of the diencephalic part of the brain. The original passage from Galen as quoted by Walker ('38) reads as follows "Cavi enim intus fuerunt, quo spiritum reciperent, sursumque usque ad cerebri ventriculum ob eandem causam pertigerunt, ubi enim uterque ventriculus anterior definit ad latera, illinc nervi optici exoriuntur, ipseque ventriculorum velut thalamus propter illos nervos extitit" (Galenus, De Usu Partium, 16. III).

2 The problem of the establishment of homologies in the central nervous system has been discussed extensively in a number of publications (see for example Kuhlenbeck, '26, '29a, '29b, '30, '33, '34, Kallen, '51, '59, Nieuwenhuys and Bodenheimer '66, Campbell and Hodos, '70).

It should be mentioned parenthetically that similarity in fibre connections is considered a very important criterion by the experimental neuroanatomists (see for example: Nauta and Karten, '70).

The search for the 'obviously similar structural plan' belongs, in part at least, to the realm of comparative neuroembryology. The relative simplicity of the embryonic nervous system invited a number of investigators to study the development of the brain. If anywhere a basic structural pattern could be brought to light, it should manifest itself during ontogenesis.

The authors who have studied the ontogenesis of the mammalian diencephalon have arrived, however, at widely divergent interpretations. These discrepancies are due to the fact that most of them studied only one single developmental phenomenon and employed this as the sole criterion of interpretation and subdivision.

Some authors (Von Kupffer, '06; Palmgren, '21; Rendahl, '24; Holmgren, '25; Bergquist, '32; Bergquist and Källén, '54) employed the early developing neuromeres as the transversally arranged basic elements of the neural tube and it is in these terms that the ontogenetic process is described by them (see fig. 1).

Bergquist ('32) and Bergquist and Källén ('54) were able to discern within the confines of the neuromeres subunits which they termed "Grundgebiete" or "Migration Areas" and which together constitute the structural plan of the brain. In the early phases of development a migration area is a region of neuroepithelium characterized by a high incidence of mitoses around a sulcus in the centre of that region. The authors reported that they could follow these regions from the neuromeric stage throughout development.

A longitudinal subdivision of the brain by a sulcus limitans into basal (motor) parts and alar (sensory) parts was worked out by His (1893[a]) for the lower brain stem. This subdivision seemed to be extended into the diencephalon by the results of a comparative study on the diencephalon of the amphibian brain

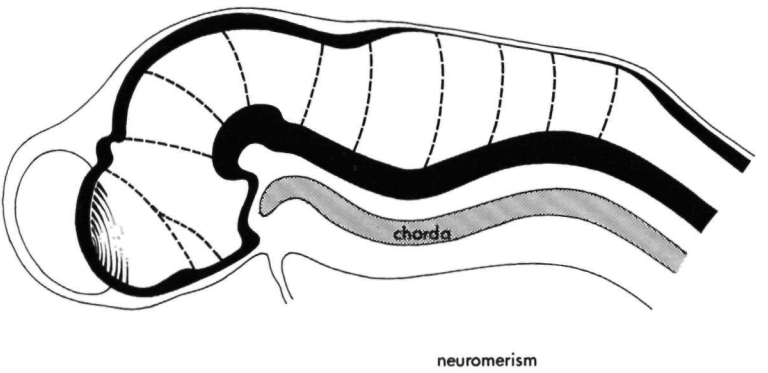


Fig. 1 Schematic representation of the structural plan of the brain based on neuromerism (Bergquist and Källén, 1954).

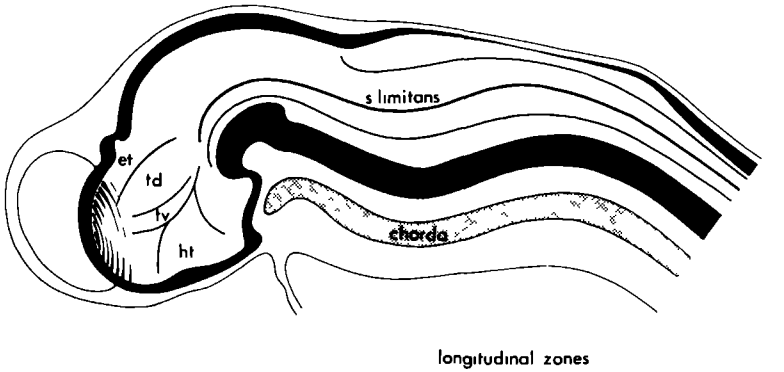


Fig 2 Schematic representation of the structural plan of the brain based on longitudinal cell columns (His, 1893a).

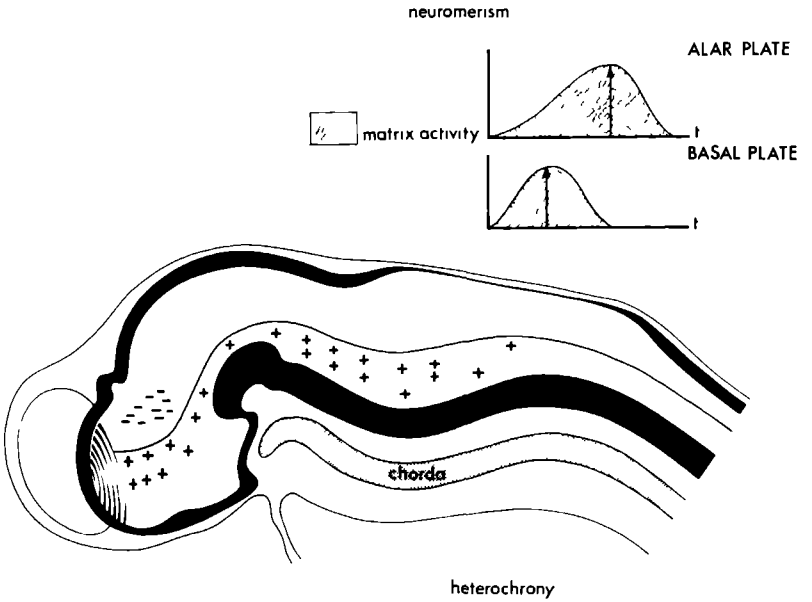


Fig. 3 Schematic representation of the structural plan of the brain based on heterochrony phenomena (Kahle, 1951, 1956).

published by Herrick in 1910. The latter author created an important role for the sulci that groove the lateral ventricular wall of the diencephalon. They formed boundaries dividing the diencephalon into four longitudinal zones arranged in superposition to one another (see fig. 2). This "four story" diencephalon, consisting of epithalamus, pars dorsalis thalami, pars ventralis thalami and hypothalamus has been recognized by a large number of investigators in representative groups of vertebrates. Droogleever Fortuyn ('12) and Kuhlenbeck

('30, '54) were among the first to provide evidence in favor of the existence of this longitudinal zonal pattern in the mammalian diencephalon. As Herrick, Kuhlenbeck considered the stories to be "Grundbestandteile" in his Bauplan (structural plan). These basic units are delimited by sulci on the ventricular surface (Kuhlenbeck, '29[b]).

The considerable difference in rate of development of the matrix in different regions of the brain has led some authors to use this phenomenon, called heterochrony, as a clue to the definition of morphological entities in the embryonic brain. Rose ('42) and Grunthal ('52) made an occasional use of this principle in their publications, but Kahle ('51, '56) was the first to employ heterochrony phenomena systematically in order to describe the development of the diencephalon and to characterize its subdivisions (see fig. 3). Following this criterion, Kahle was able to delimit the same longitudinal zones within the diencephalon as did Herrick and Kuhlenbeck, with the reservation that within the hypothalamus he considered the dorsal part a separate zone, which he called subthalamus.

Although the study of fibre connections in the analysis of the adult brain is of great importance and although they have been employed by many authors for the establishment of homologies, systematic studies on the ontogenesis of fibre connections are only few in number (Windle, '32[a], '32[b], '33, '35; Windle and Baxter, '36, Lyser, '66). The validity of the pattern in which fibre connections develop, as a clue to the subdivision of the mammalian diencephalon, has never been systematically investigated. Senn ('68, '70), however, has recently introduced the study of fibre configurations in the ontogenesis of the brain. In his studies he took the very distinct fibre laminae which are present in the roof of the midbrain of reptiles as a starting point and he tried to extend this lamination pattern also over other parts of the brain, including the diencephalon. A specific point in this approach is, that Senn completely disregarded the origin and the ending of the fibres but only considered their tendency to constitute layers. It is interesting to note that according to Senn the fibre layers just mentioned can also be distinguished in the pretectal area and in the diencephalon of reptiles. No comparable study has been published on the mammalian brain thus far.

From this thematic survey of the literature the study of the ontogenesis of the diencephalon appears as a battleground where conflicting ideas on the nature of the basic structural plan of the brain clash. Whereas His, Herrick, Kuhlenbeck and Kahle advocated a subdivision of the diencephalon into horizontally arranged zones, the subdivision of Bergquist and Källén is based on the presence of transversely oriented units. In the work of Senn, that thus far has not been extended to mammals, a third means of subdivision is employed, *i.e.*, the presence of tangentially oriented alternating cell- and fibre-layers.

In view of the obvious discrepancies just recorded, a study of the relations between the various phenomena used as criteria of subdivision by the different authors seems warranted.

In order to achieve this goal, namely to test the validity of and the relations between the various criteria used in the literature on the embryology of the mammalian diencephalon, it was necessary to first verify the very existence of all the phenomena mentioned, in one and the same species. For that purpose an analysis was performed of the ontogenesis of the diencephalon in a mammal which possesses a relatively simple brain, the Chinese hamster.

After a survey of the techniques and methods used in this study (Chapter II) a description will be given of the transformations of the prosencephalic midline structures during development (Chapter III). This chapter is intended to provide the reader with a terminological background and with a framework, within which the following embryological discussions should be understood. Chapter IV communicates the results of an investigation into the presence of neuromeres at the earliest phases of development of the neural tube. The question is also raised there, whether and if so to what extent, the fate of these neuromeres can be traced in the adult brain, or whether they are merely phenomena of a temporary nature. In chapter V the changes of the ventricular relief are studied as they can be observed throughout development from the early neuromeric stages into the adult diencephalic configuration, where the boundary sulci of Herrick and Kuhlenbeck can be demonstrated.

In chapter VI the heterochrony phenomena are studied which become manifest when the developmental state of the matrix is observed. In order to facilitate the description of the events occuring in the neural tube's wall during development, the natural history of the neuroepithelium was staged into nine phases. Using this code, the functional state of the neuroepithelium in each particular stage was mapped out on the ventricular surface. Thus heterochrony phenomena within the diencephalon are readily illustrated and can be compared to the other phenomena under consideration. The formation and differentiation of the mantle layer is the subject of chapter VII. In this part of the study the development of the grisea is studied together with the appearance of the main 'fibre streams'. It is supplemented by an autoradiographic investigation of the sequence of origin of the grisea which are observed in the histologically stained series (Chapter VIII).

These analytical chapters are followed by a synthesis (Chapter IX) in which the literature on the development of the mammalian diencephalon is evaluated. The results of the present study are reviewed and the interrelations are discussed between morphogenetic and histogenetic phenomena. (For a specification of these phenomena the reader is referred to table I). Further, this chapter presents a general picture of the structural plan of the diencephalon under study, and attempts to solve a number of the controversies extant in the literature.

MORPHOGENESIS		HISTOGENESIS	
		HETEROCHRONY	DIFFERENTIATION
neuromere derived sulci (chapter IV)		matrix development phases 1 to 9 (chapter VI)	mantlelayer formation (chapter VII, VIII)
interneuromeric sulci (chapter V)			
sulci of different origin (chapter V)			
expression	pattern ← (chapter IV, V)	process	pattern → (chapter VI, VII, VIII) expression

Table I. Survey of the developmental phenomena which are the object of study in later chapters.

CHAPTER II

MATERIALS, TECHNIQUES, METHOD

ANIMALS

The Chinese hamster was introduced into neuro-anatomical research by Droogleever Fortuyn ('27) and recently a bibliography on this laboratory animal has appeared (Weihe and Isenbügel, '70).

The choice of the Chinese hamster as a test-object in this study was determined by the desirability in mammalian embryological work, to be informed as accurately as possible on the postcoital age of the embryos. The breeding method used at the Central Animal Laboratory (Van Gaalen, '63) permitted us to limit the period of exposure of the female to the male's overtures to 3 hours. In this way the uncertainty about the postcoital age of embryos could be reduced to plus or minus one and a half hours, *i.e.*, considerably shorter than in other rodents.

The gestation period of the Chinese hamster lasts approximately 21 days. The age of the embryo was determined according to the number of days that had elapsed after coitus had taken place; thus the age at the day of mating was recorded as E_0 (embryonic day 0), at the next day as E_1 etc..

Empirically the moment of closure of the neural tube was determined. This important event in early neurogenesis appeared to occur at approximately 10 days, 18 hours postcoitum. This observation is in accord with the findings of Niimi *e.a.* ('61) in the mouse and Adelman ('25) in the rat who pointed out that embryonic development in these animals progresses very slowly in the first half of intrauterine life. The same tendency was observed in rabbit embryos by Bianchi ('09).

A variation between littermates was observed for rodentia by Ziehen ('06). Tello ('34), however, argued that, at least in the mouse, this difference was more a quantitative than a qualitative one. On the basis of our experience in the Chinese hamster embryo it can be stated here succinctly that no variation in development between littermates was observed that exceeded a corresponding time period of approximately 6 hours.

NORMAL MATERIAL

Material was collected consisting of embryos ranging from embryonic day 10 (E_{10}) to embryonic day 21 (E_{21}) and of brains of postnatal animals 3, 6, 9 and 100 days of age (see table II). Fixation was performed by immersion in either

Postcoital age	Total number of Embryos studied[1])	Number of Embryos reconstructed	Number of nests
9½	9		3
10	19		6
10¼	8		4
10½	9		3
10¾	6		3
11	7		3
11¼	3		2
11½	4		3
12	17	4	3
12½	8		2
13	21	4	6
13½	10		3
14	17	4	6
15	11	4	4
16	18	3	7
17	13	4	4
18	20	4	5
Postnatal age			
0	11	1	
3	3	1	
100	3	1	

Table II: The material on which the present study is based. The average number of embryos in each nest varies. In literature it is given as 5 (Yerganian, '58, '67); in our material the median number of littermates was 6 (van Gaalen, '63).

1) In each litter a sagittal and two transversal series were prepared. The directions of sectioning of the transversal series were aimed to be perpendicular to each other.

Carnoy's or Bouin's fluid (Romeis, '68). Up to the E_{13}-stage fixation took place in utero because of the vulnerability of the embryo. After 6 hours of fixation the embryo was removed under the dissection microscope. Postnatal animals were perfused with Bouin's fluid; after dehydration and embedding in paraffin the

embryonic material was sectioned at 7 or 10 micra and stained according to Mayer's modification of the haematoxyline and eosine techniques. (Romeis, '68). Postnatal animals were stained with a modification of the Nissl technique (Romeis, '68) and with Klüver-Barrera's stain (Klüver and Barrera, '53). In addition to the material mentioned above the author had at his disposal a complete series of Bodian-stained embryos in order to verify the existence of fibre tracts.

EXPERIMENTAL MATERIAL

The autoradiographic part of the study was performed on embryos which received a dose of tritiated thymidine either directly via an intra-uterine injection or indirectly by way of an injection into the peritoneal cavity of the mother. The intra-uterine dose was fixed according to age, E_{10}, E_{11} and E_{12} receiving 10 μC/embryo; E_{13} and E_{14} 15 μC/embryo; E_{15} and E_{16} 20 μC/embryo; E_{17} and E_{18} 25 μC/embryo. The intra-peritoneal dose was calculated as 5 μC/g maternal body weight[3]). Embryos were fixed in Carnoy's solution, dehydrated, embedded in paraffin, sectioned in one of the cardinal planes at 7 micra and mounted on slides. Under safe lighting these slides were coated with a radio-sensitive emulsion (Ilford G 5 or K 5 emulsions) by the dipping technique as described by Berry and Rogers ('65). The slides were kept in a refrigerator at 4°C and protected against incident radiation by a lead box for approximately three weeks. Subsequent development took place with an amidol developer at 15°C during 10 minutes. The sections were stained after development with either toluidineblue or haematoxyline-eosine[4]).

The embryos, having received an injection at different days of developmental age, were allowed to survive for a varying period. In table III a summary is presented of the design of the experimental part of the study recording the age of the embryos at the time of injection and the survival time of the animals after injection.

TECHNIQUES OF RECONSTRUCTION

In order to visualize the results obtained in this study it was thought necessary to make reconstructions of the embryonic brains that were investigated.

3. The doses of the intra-uterine injections are adapted from Berry and Rogers ('65). The doses of intra-peritoneal injections are derived from the work of Altman ('66) and Angevine ('65, '69).

4. A number of thorough publications on the technique of autoradiography exists (Rogers, '67; Sidman, '70). The author received a travelling fellowship from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) to visit Dr. Rogers in Oxford in order to learn the autoradiographic technique. He wishes to express his gratitude for the hospitality extended to him by the Staff of the Department of Human Anatomy in Oxford in 1967.

age of the embryo at day of injection	Survival time after injection								
	6h	1 day	2d	3d	4d	5d	6d	7d	100PN
10						x			X
11		x		x		x		X	X
12	x	x		x		x	X		X
13	x	x	x	x	x	X			X
14	x	x	x	x	X	x			X
15	x	x	x	X		x			X
16	x	x	X	x	x				X
17	x	X	x	x					X
18	X	x	x	x					X

Table III: The design of the tritiated thymidine experiment. Each cross represents a pregnant Chinese hamster, injected at a specific postcoital time during pregnancy. The mother was allowed to survive up to the time that can be deducted from the table. From each embryonic day of injection, one litter was allowed to survive to the adult stage (PN 100).
After a preliminary study of the whole material only the animals of ages F_{18} and PN_{100} (X in the table) were included in the study reported in chapter VIII.

Most illustrations were produced by means of graphic reconstructioning. This procedure involves essentially three steps: First, from a bilaterally symmetric series of transverse sections every 5th or 10th section is drawn on translucent paper; the midline of every particular section is drawn and the structures to be reconstructed are projected and plotted on this midline. Second, with the help of sagittal series of the same age the plane of sectioning of the transverse series is determined. Then a reconstruction is made of its median outline. Third, on this midsagittal plane the points that are projected and plotted on the midline in each particular section are copied. A picture then arises of the configuration of the entities under consideration.

The most critical step in this procedure is the reconstruction of the median outline of the third ventricle. In order to obtain insight in the configuration of this formation a number of embryos was cleared in methylbenzoate and photographed. Additional information was provided by embryos that were embedded in paraffin, sectioned sagittally up to the midline, then deparafinated and photographed in alcohol. The latter technique makes it possible to throw a glance at the ventricular surface of the brain. The information thus obtained, together with data from sagittally sectioned histological series, allows the reconstruction of an ideal median section of the stage under consideration.

Employing the thickness of the sections of the transverse series as one datum and the presence of median landmarks both in the transverse sections and in the ideal median section as another datum it becomes possible to determine the exact direction of sectioning of the transverse series. Subsequently the real midline configuration of the transverse series is reconstructed, using the ideal midline configuration as a blue print.

It should be appreciated that the technique of graphic reconstruction has some limitations; the compression of the tissue caused by the cutting of the sections may alter the configuration of the midline structures of the embryo under study. Furthermore, the bilateral symmetry of the transversely sectioned series is a prerequisite in order to obtain reliable reconstructions of laterally located structures.

A three dimensional reconstruction of the eighteen day embryo's diencephalic mantle layer configuration was worked out (under control by sagittally sectioned series) at a magnification of 100 x, using styrofoam® plates of a thickness of 5 mm as the basic material[5]). This is a commercially available material and can be easily cut with the help of a hot wire; large scale reconstructions can be obtained readily (Richter, '65; Fernández-Ortega, '68).

METHOD

The methodological background and the conceptual basis that form the setting for a discussion of the results obtained in the analytical chapters of this study have to be elaborated to some extent at this point. The aim of this study is to unravel the complexity of the developmental changes of the diencephalon within one single species. It is attempted to solve the controversies that exist in literature, and that are caused by the rigid use of one single phenomenon as the sole criterion on which the subdivision of the diencephalon can be based, to the exclusion of all other possible criteria.

It may be expected therefore, that the present study will yield information on the relations between the structural plans that are presented by the different authors.

An attribute of the neural tube, that must be accounted for in every structural plan of the developing nervous system is, in our opinion, the preservation of the continuity and integrity of the internal and external surface of the neural tube's wall. No new continuities develop (with the exception of the massa intermedia) so far as the diencephalon is concerned. As the external and internal surfaces have a histologically circumscript relationship it must be possible to describe changes in the configuration of the neural tube during development as topological transformations of the originally simple neural tube. These transformations are closely related to histogenetic events in the neural tube's wall and

5. This material is called Steropore® or Polystyrol® on the European continent.

it is this aspect of the development of the central nervous system that recently has become more accessible for investigation. Present-day techniques make it possible to determine the moment of differentiation of a neuron. This moment can be recorded in the tritiated thymidine experiment. By using several survival times it is possible to trace the way along which a neuron has migrated from the stretch of neuroepithelium where it originated to its ultimate position. This fact and the dynamic concepts that have sprung from studies on the natural history of the neuroepithelium profoundly influenced our understanding of the developmental processes in the central nervous system.

It is against this conceptual horizon that the results from the analytical chapters have to be considered and will be discussed.

CHAPTER III

TERMINOLOGICAL AND MORPHOLOGICAL ORIENTATION

The aims of this introductory chapter are to provide the reader with a terminological background and to present a general orientation concerning the morphological transformations that take place during development. Although this study focusses upon the developmental events taking place after the moment of closure of the anterior neuropore, it is relevant to discuss some data on the early development first. In the second part of this chapter certain landmarks or invariants are introduced which enable us to give a description of the transformations of the structures in the median plane. Together these landmarks function as a frame of reference for the description of the prosencephalic region of the brain. The transformations are discussed using a series of drawings that show the midline structures reconstructed from sagittally sectioned series of the brain in various stages of development (see fig. 4).

SOME REMARKS ON THE GENERAL MORPHOGENESIS OF THE CENTRAL NERVOUS SYSTEM

After the formation of the germ layers a neural plate is induced in the ectoderm. This early embryogenetic event is caused by an influence from the archenteron roof that triggers the neural differentiation of the overlying epithelium (Spemann, '36; Lehmann, '38, '41, '50; Nieuwkoop, '47, '52; Toivonen, '40, '49). During further development, the lateral margins of the neural plate raise, thus altering the plate into a neural groove. Eventually the two lateral lips of the neural groove meet dorsally in the midline and fuse; this marks the formation of the neural tube. This structure gradually becomes detached from the overlying epidermis and sinks 'en masse', relatively at least, into the mesoderm of the embryo. According to classical embryology the fusion of the lateral margins is initiated at the future mesencephalic level (Mihalkovicz, 1877; Kingsbury, '22) or immediately in front of the trigeminal anlage and proceeds from this region of earliest closure by one continuous process in both the rostral and the caudal direction as classical embryology learns. Schulte and Tilney ('15), however, reported its initial incidence at several separate points of the neuraxis at the same moment.

The wide openings at either end of the first point of fusion are called anterior and posterior neuropore respectively and become smaller and smaller as fusion proceeds. As the ultimate sites of closure of the neural tube they possess

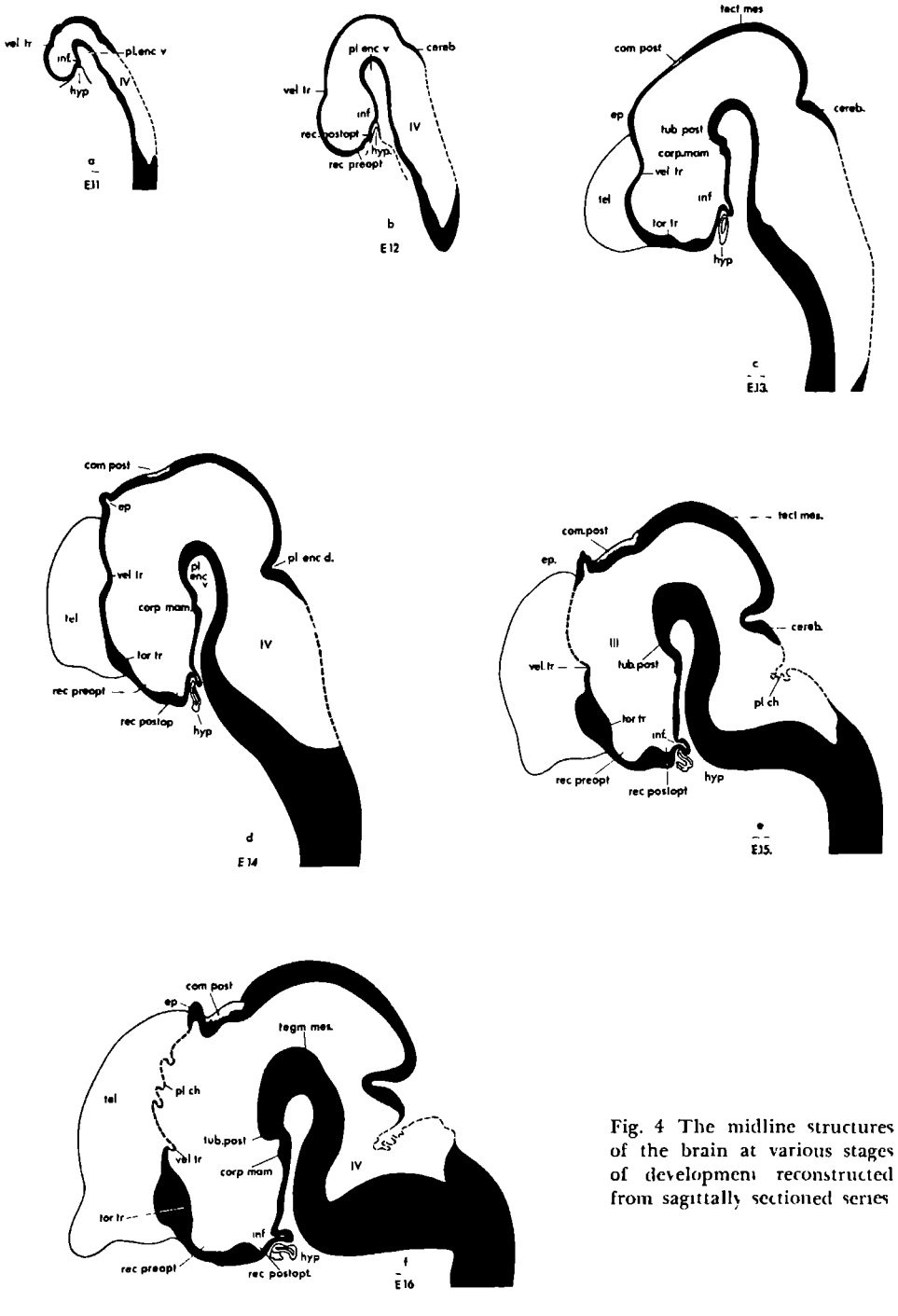
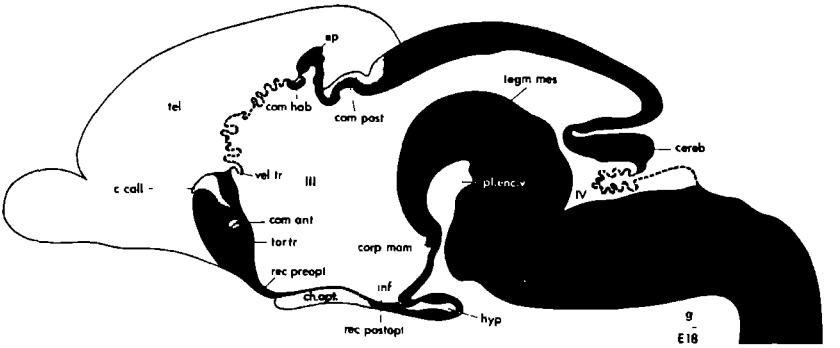


Fig. 4 The midline structures of the brain at various stages of development reconstructed from sagittally sectioned series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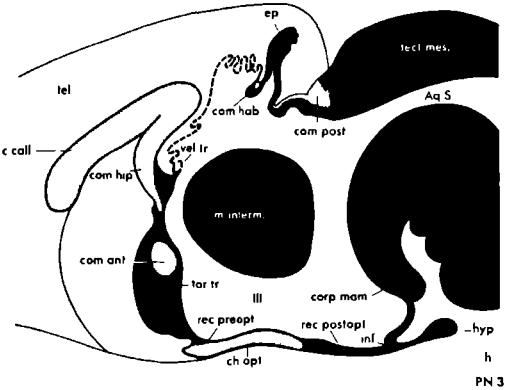
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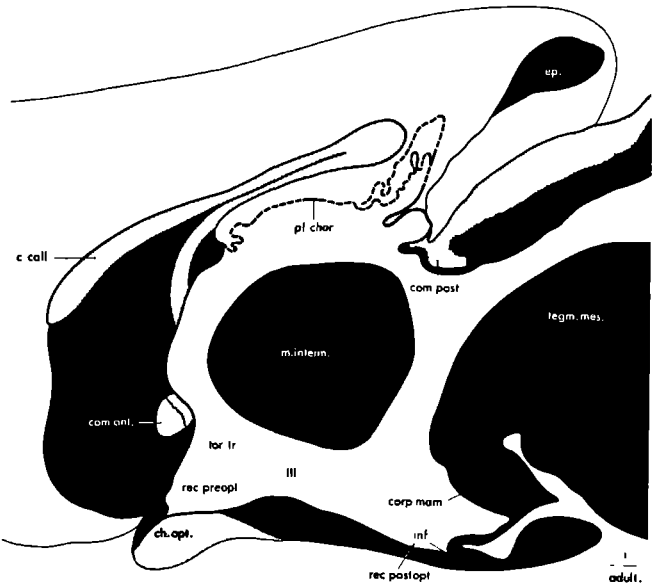
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considerable morphological significance. As far as the neuroporus anterior *s.s.* is concerned, the final point of closure is reported to leave a remnant in certain species, the neuroporic recess. Literature is quite frugal in respect to the number of exact descriptions of this landmark in neurogenesis. Its supposed position, however, is frequently used in order to describe the ending of a central brain axis and thus the localisation of the anterior neuropore *s.s.* remained the object of much debate (Tandler and Kantor, '07; Johnston, '09; Hines, '22).

After the closure of the anterior neuropore, the rostralmost part of the young neural tube can be divided in two parts, the archencephalon and the deuterencephalon (Von Kupffer, '06). The deuterencephalon is situated dorsal to the chorda dorsalis; hence it is called the epichordal part of the brain tube, whereas the archencephalon occupies a prechordal position. The epichordal part of the neural tube can be described as composed of two lateral plates, which furnish the neurons of the brain stem and spinal cord by their proliferative activity and subsequent differentiation (His, 1888). The lateral plates are connected by a dorsal roof plate and a ventral floor plate (Burckhardt, 1894) both of which are later devoid of neuroblasts and composed of ependymal cells (Kingsbury '20). The composition of the archencephalon as compared to the deuterencephalon can be expressed in terms of the deuterencephalic components. For that purpose the neural tube can be thought of as unrolled and unfolded and the extent of its different components can be indicated in a simplified diagram illustrating the fundamental morphological plan of the brain. Several opinions on this subject were expressed in literature (see fig. 5).

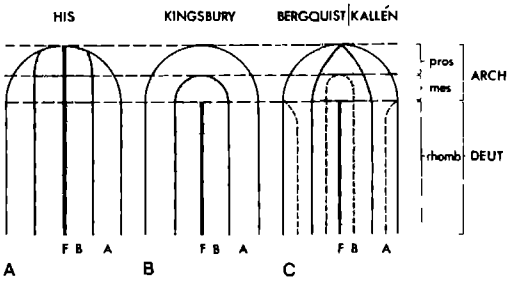


Fig. 5 The morphological plan of the brain according to A) His (1893b), B) Kingsbury (1922) and C) Bergquist and Kallén (1954). The plans are superimposed on diagrammatic drawings of the neural plate. (Indices within the figure: A = alar plate; B = basal plate; F = floor plate).

His (1893b) extended the floor plate to the rostral margin of the neural plate (fig. 5a). Kingsbury, however, in a series of elucidating papers ('20, '22, '30, '32, '34) demonstrated convincingly the extent of the floor plate as limited to the

deuterencephalic part of the brain (fig. 5b). Thus, to his opinion, the brain consisted of two symmetric halves (the lateral plates), connected rostrally by a primary continuity and otherwise by a median structure, the future 'floor plate'. The extent of the functional subdivisions of the lateral plates (lamina alaris and lamina basalis, the sensory and motor primordial columns) as conceived by His (1888) is indicated in the diagrams. In a third diagram (fig 5c) the subdivisions of the lateral plates into visceral and somatic columns (Johnston, '02) as forwarded by Bergquist and Källén ('54) is indicated. It represents in several respects a modification of the scheme proposed by Kingsbury. The most salient difference between the concepts of Bergquist and Källén on the one hand and Kingsbury on the other hand is that according to the former, only the basal plates are directly continuous around the most rostral point of the floor plate, whereas according to Kingsbury, both basal and alar plates show such a continuity.

The question of the localisation of several landmarks in these morphological plans and of the extent of the roof plate will be taken up later on in this chapter.

The cerebral flexures

As early as the neural plate stage, a dorsally concave flexure at the level of the future spinal cord, and a ventrally concave curvature in the area between the future archencephalic and deuterencephalic regions is observed. The latter which remains a characteristic of the neural tube throughout development, was termed flexura cephalica by His (1888) and plica encephali ventralis by Von Kupffer ('06). The midbrain or mesencephalon comes into existence on top of the plica encephali ventralis, at the junction of archencephalon and deuterencephalon, and is separated from the prosencephalon in front and from the rhombencephalon behind by two external fissures. Hence the cerebral tube begins to resemble a series of vesicles, as was already observed by Von Baer (1828) in the chick. Later on, the rhombencephalic part of the neural tube shows a ventrally convex pontine flexure that increases gradually, *pari passu* with the development of a dorsally convex cervical flexure, located between the rhombencephalon and the spinal cord.

Both prosencephalon and rhombencephalon eventually show a subdivision into two parts, and thus the classical five brain vesicles develop, *i.e.*, telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon (Huxley, 1871).

Terminological considerations

The complex configuration of the neural tube presents some descriptive difficulties regarding orientation. In the later stages a transverse plane,

perpendicular to the central brain axis, will be different for each brain segment, when related to the embryo as a whole. Most descriptions in neuroanatomy, therefore, are expressed in relation to the morphological position, a convention by which the neural tube is unrolled, thus stretching its central brain axis. The adjectives rostral and caudal are used to indicate the relative position along this axis. The structures which are located more toward the convexity of the brain, are called dorsal, and those that have a position more toward the concavity (as related to the central brain axis in the morphological transverse plane) are called basal or ventral. All of these terms are used irrespective of the topographic position in the body and only with reference to the neural tube itself.

Orientation in the forebrain

The question of the ending of the central brain axis rostrally in the prosencephalon presents a crucial problem in terminology of the forebrain. Convention is apt to locate this point in the definitive anterior neuropore, the ultimate point of closure in the rostral part of the neural tube. A difference of opinion concerning the localisation of this neuroporus anterior *s.s.*, however, exists. His (1893[b]) held the view that the Basilarleiste (corresponding to the recessus postopticus in later stages) formed the rostralmost ridge of the open neural plate. According to him the closure of the anterior neuropore progresses in two directions; from the first place of fusion of the lateral margins of the neural plate dorsally a roof plate forms in a rostral direction. The ventral lips of the anterior neuropore also fuse, thus forming a terminal suture ("das frontales Endnaht"). In His's opinion the two processes of fusion meet in the recessus neuroporicus. Thus a terminal wall is formed reaching from the recessus post-opticus (Basilarleiste) to the recessus neuroporicus. Nowadays, the rostral border of the neural plate is universally agreed upon as corresponding to the optic chiasm in the adult brain ("This is a matter of fact, not of interpretation", Johnston, '09), and the terminal wall should accordingly reach from the chiasm to the neuroporic recess. This part of the median wall of the prosencephalon, rostral and dorsal to the optic chiasm maintains a key position in a proper understanding of the morphology of the brain, the essential morphological relationships being completely preserved throughout the vertebrate series (Johnston, '09). It constitutes the rostral extreme of the neural tube. In later stages, it is incorporated in the most rostral unevaginated extreme of the telencephalon, *i.e.*, the telencephalon medium or telencephalon impar. Thus a part of the median ventricular lumen is distinguished as telencephalic, bounded in front by the structures of the telencephalon medium (M. Hines, '22), as opposed to the remaining, diencephalic part of the ventricle. The telodiencephalic boundary is defined by a constriction of the brain tube, that crosses the midline dorsally as the velum transversum. This structure retains its epithelial character

throughout development and appears in sagittally sectioned series as an indentation of the dorsal wall of the tube. Ventrally the telodiencephalic border, according to some authors at least (Mihalkovicz, 1877; Meek, '07, '09, '10; Kuhlenbeck, '54, '56) crosses the midline in the torus transversus, a thickening in the rostral wall of the prosencephalon, in which the anterior commissure will develop. For the sake of a clear terminology we will conform to usage and situate the terminal point of the central brain axis somewhere between torus transversus and velum transversum, although there are some reasons to question the validity of this convention (as M. Hines, ('22) puts it: 'Since the last point of closure may lie anywhere, for ought we know, between the recessus preopticus and the velum transversum'). In relation to this problematic localisation of the anterior neuropore stands the unsolved question of the definition of the lamina terminalis. For a discussion the reader is referred to Tandler and Kantor ('07) and Hines ('22). Here it is stated succinctly, that in the following pages the term lamina terminalis will be used for the stretch of neuroepithelium reaching from the optic chiasm to the last point of closure (as assumed above) thus leaving a lamina supraneuroporica between the neuropore and the velum transversum (Johnston, '13; Bailey, '15).

THE TRANSFORMATION OF THE PROSENCEPHALIC MIDLINE CONFIGURATION DURING DEVELOPMENT

As is clear from the preceding pages, the median structures of the prosencephalon do not include any floor plate derivatives. The basal prosencephalic wall consists of the unpaired, primary continuous prechordal neural plate and, in front of this, of the fused basal margins of the anterior neuropore. Dorsal to the assumed point of neuroporic closure, the roof plate extends caudally. Generalizing it can be stated that these median structures are modified during ontogenesis in two possible ways; either they are thinned out to become telae choroideae, or they increase in volume, thus providing opportunities for commissural and decussating fibre pathways to pass from one half of the brain to the other. The midline structures can be considered as invariants or reference points as their mutual relationship during development does not show any topological alterations. Thus they are landmarks that together form a skeleton. This skeleton can be followed, from the moment it appears through the entire course of development up to the adult stage even though transformations in the midline configuration are taking place.

The study of the changes in the midline configuration has the advantage of reducing the three dimensional growth process within the confines of this preliminary description into a two dimensional picture that allows a better survey of the transformations taking place. In order to provide an overall frame of reference, a series of drawings was produced showing these midline structures

reconstructed from sagittally sectioned series of brains in various stages of development (see fig. 4).

The landmarks

The landmarks which are recognisable at the E_{11}-stage (fig. 4a) are: the plica encephali ventralis corresponding to the flexura cephalica, and the infundibulum that is characterized by the strong connection between the ectodermal lining of the stomodaeum (hyp.) and the neuroepithelium; dorsally the velum transversum is developing.

At E_{12} a flexura pontina is indicated in the base of the fourth ventricle (fig. 4b). In the prosencephalon a recessus postopticus and a recessus preopticus show up as indentations of the basal wall. Their relations with the optic evagination vary according to the developmental stage; the recessus postopticus is connected with the optic vesicle in early phases by a sulcus opticus primitivus that is lost later on, when the preoptic recess is connected with the eye stalk by a secondary sulcus opticus. These relationships were established by Johnston ('09) and are mentioned here explicitly as the early recessus postopticus is easily confused with the infundibular anlage.

In the E_{13} brain the stretch of neuroepithelium between preoptic and postoptic recess is more clearly recognisable as the presumptive chiasma opticum. Caudal to the infundibulum the primordium of the corpus mamillare and the tuberculum posterius are indicated. An inward prominence located rostral and dorsal to the preoptic recess is recognised as the torus transversus, *i.e.*, the basal point of crossing between the telodiencephalic border and the midline. In the dorsal wall of the brain tube the epiphyseal anlage and the primordial commissura posterior are present. Hence already in the E_{13} stage the major landmarks characterizing the ventricular outline of the diencephalon are established and they can be followed right up to the adult stage.

An event taking place in subsequent stages of development is the modification of certain midline structures (already alluded to) consisting of either a thinning out or an increase in volume. Thus, in E_{15} and E_{16} (fig. 4e and f) the portion of the diencephalic roof situated between velum transversum and epiphyseal anlage develops into the plexus chorioideus ventriculi tertii. In the meantime the epiphysis seems to be displaced in the caudal direction towards the commissura posterior incorporating the intervening part of the ventricular lining in its stalk.

In E_{18} (fig. 4g) a further increase of the optic chiasm can be observed and in the torus transversus a commissura anterior has developed. In the dorsal part of the telencephalon medium another telencephalic commissure has come into existence; it is the primordium of the corpus callosum and of the commissura hippocampi; by its enormous increase in volume this commissure will become

a prominent feature of the prosencephalon (see fig. 4g, h and i). Just in front of the epiphyseal anlage a commissura habenularum is now visible.

Beyond the E_{18}-stage the only additional characteristic feature of development is the fusion of the dorsal thalamic cell masses giving origin to the massa intermedia (PN_3, PN_{100}, fig. 4h and i).

The transformations

With all of these landmarks at hand it is possible to give a concise description of the alterations taking place during development in the outline of the prosencephalic median ventricle. It has been pointed out already, that these landmarks do not appear at the same time and that the structures, although invariant with respect to their topological position, do show considerable shifts with regard to their topographical position.

First we will consider the changes occuring in the structures that constitute the basal median wall of the prosencephalon; here, from the anterior neuropore backwards it is possible to discern the lamina terminalis, the preoptic recess, the optic chiasm, the postoptic recess, the lamina postoptica, the infundibulum, the lamina praemamillaris and the corpus mamillare.

The lower part of the lamina terminalis, in front of the preoptic recess, gradually raises from being more or less in line with the primordial chiasma opticum at E_{13} (fig. 4c); at the E_{15}-stage (fig. 4e) it passes through a position of circa forty-five degrees and eventually forms a ninety degrees angle with the optic chiasm in the adult stage (fig. 4i).

The lamina postoptica, extending from the postoptic recess towards the infundibular stalk, at first forms an obtuse angle with the optic chiasm (E_{13}, fig. 4c) which sharpens to about ninety degrees at E_{14} (fig. 4d) and later on comes in line with the lengthening chiasm. By this process the direction of the infundibular stalk is changed from a more or less rostral one (in E_{13}) into a caudal one in later stages (see *e.g.* PN_{100}, fig. 4i). The lamina praemamillaris stretches between infundibular stalk and corpus mamillare. At first it makes an obtuse angle with the postoptic lamina. In E_{14} (fig. 4d) however, it forms a rectilinear continuation of the latter structure, caudal to the interruption caused by the infundibular stalk. The length of the lamina praemamillaris increases in E_{15} but later on it is relatively reduced once again by the development of the structures located caudal to it. There, the corpus mamillare, from being located in a rather 'high' position in the plica encephali ventralis, is considerably lowered, presumably because of the progressive thickening of the mesencephalic tegmentum (see E_{13}, fig. 4c and E_{18}, fig. 4g). A striking feature in this caudalmost part of the diencephalic basis, is the appearance of the tuberculum posterius in the E_{16} stage, probably caused by the tegmentum's development.

Now, we will turn to a description of the changes that occur during ontogenesis

in the median dorsal wall of the prosencephalic part of the brain; here it is possible to discern, from the anterior neuropore backward, the lamina supraneuroporica, the velum transversum, the plexus chorioideus ventriculi tertii, the commissura habenularum, the epiphysis and the commissura posterior.

At first (E_{13}, fig. 4c) the direction of the prosencephalic roof, continuing rostrally beyond the velum transversum in the dorsal part of the telencephalon medium, is mainly perpendicular to the chiasma opticum. The epiphyseal anlage, in the E_{14}-stage, is shifting towards the posterior commissure and this relative displacement continues during the next two stages (E_{15}, E_{16}); in the meantime the part of the roof structures located between velum transversum and epiphyseal anlage becomes thinned out into a membraneous layer. The elongation of the terminal wall and its rising which has been described in the preceding paragraph, results in a position of the dorsal diencephalic wall more or less parallel to the optic chiasm in the adult stage (PN_{100}, fig. 4i).

SUMMARY AND CONCLUSION

The changes in the midline configuration that were described thus far, no doubt are interdependent. The midline structures, of course, undergo a profound influence from the events occuring in the lateral walls of the neural tube, but these events have intentionally been left out of consideration in this preliminary reconnaissance.

When an attempt is made to combine the results obtained from the study of the transformations into an overall picture of the changing midline configuration, at first sight attention is directed to the changing relationships between the cerebral flexures. Initially the flexura cephalica increases considerably (E_{11}, E_{12}). In later stages the pontine and cervical flexures are added (E_{12}–E_{15}), the cephalic flexure remaining approximately constant. Later on, this cephalic flexure (as measured from the position of the prosencephalic longitudinal main axis), diminishes, and closer analysis reveals a narrow relationship between this 'stretching' of the brain and changes in the mesencephalic wall. In the mesencephalon, the tectum seems to be displaced in a caudal direction when compared to the position of the tegmentum. The latter structure seems to move in the opposite way, *i.e.*, in a relatively rostral direction, thus becoming part of the anterior limb of the plica encephali ventralis. Hereby the mamillary anlage is transfered downwardly, *i.e.*, towards the lower end of the plica encephali ventralis. This event may be a major factor in the further morphogenesis of the diencephalic floor that is effected as a consequence. The optic chiasm and the postoptic and praemamillary laminae are transformed into a more or less straight part of the diencephalic wall that commonly is regarded as forming the basis diencephali in the adult brain. This fact together with the intrinsic lengthening of the different components of the ventricular outline results in a

transformation that bears a certain resemblance with a rotation movement around a transverse axis not unlike the movement of a transport band at its turning point (cf. Luyendijk, '44). In this rotation movement, all the diencephalic landmarks discussed are involved. The progress of this movement is particularly clearly illustrated by the fact, already pointed out by Gilbert ('34), that during development, the infundibulum 'passes' the tip of the hypophyseal stalk. The latter must be considered a fixed point, possessing strong connections to surrounding structures.

Haller ('29) and Grünthal ('52) have demonstrated that during development the extent of the cephalic flexure gradually decreases; it will be clear that this phenomenon can be explained by the rotatory mechanism as described above, which affects the direction of the longitudinal brain axis as well.

The general form of the prosencephalic ventricular lumen as seen in the lateral aspect, is modified during development by the transformations described above from a slender and oblong outline in the E_{16}-stage into the more compact 'square' permanent configuration seen in later stages (E_{18}, PN_3) as well as in the adult (PN_{100}).

CHAPTER IV

NEUROMERISM

INTRODUCTION

The development of neuromeres

Most modern textbooks on neuroanatomy in their description of the early phases of the developing brain distinguish a two-, three- and five-vesicle stage as referred to above (chapter III). This way of subdividing the brain is based on early observations (Von Baer, 1837; Huxley, 1871; Mihalkovicz, 1877), and was used by His (1893a) as a foundation for his proposed nomenclature incorporated in the B.N.A. in 1895 (His, 1895).

On closer scrutiny, however, the validity of this model within the scope of comparative neuroanatomy can be seriously called into question. A large number of authors, among them Orr (1887), McClure (1891), Locy (1894), Froriep (1892), Meek ('07, '09, '10), Haller ('29) and Streeter ('33), mentioned the occurrence of a larger number of vesicle-like structures or 'neuromeres' in the developing brain. They all concur in the description of an increase in number of these neuromeres as development proceeds, but a wide variation of opinion prevails as to both the correspondence of the number of neuromeres to each of the five vesicles in His's subdivision, and to the interpretation of the morphological value of the structures in question. Unanimity exists among these authors concerning the secondary character of the five-vesicle-model when compared to the primary phenomenon of neuromerism. According to them the secondary brain vesicles are of minor morphological importance. Since the developmental events just touched upon are of paramount importance for the present study it is necessary to review the literature at this juncture. The term 'neuromere' was coined by Orr (1887). The structures mentioned by that name were described earlier by Mihalkovicz (1877) as 'Falten'. Orr, in his study of lizard embryos, formulated a number of criteria which a neuromere should fulfill in order to apply to this designation. He is quoted here in full: ".... there have appeared in the lateral walls of the hindbrain and the region of the thalamencephalon a number of symmetrical constrictions, giving the walls in horizontal, longitudinal, section an undulated appearance.... I have adopted for them the word 'neuromeres' .... Each neuromere is separated from its neighbours by an external dorso-ventral constriction, and opposite this an internal sharp dorso-ventral ridge, — so that each neuromere (*i.e.*, one lateral half of each) appears as a small arc

of a circle. The constrictions are exactly opposite on each side of the brain". He then gives a histological description of the arrangement of the cells in the neuromeres at a very early stage: "The elongated cells are placed radially to the inner curved surface of the neuromere. The nuclei are generally nearer the outer surface, and approach the inner surface only toward the apex of the ridge. On the line between the apex of the internal ridge and the pit of the external depression, the cells of adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another neuromere. This definition of adjacent neuromeres presents, in some sections, the appearance of a septum extending from the pit of the external depression to the summit of the internal ridge .... This septum may be nothing else than those parts of cell-walls which form the boundary line of the neuromeres, and which are made conspicuous by lying in a straight line" (Orr, 1887, p. 334-335).

The neuromeres were subsequently denominated under various terms. McClure (1891) introduced the term 'encephalomeres' by which are mentioned the neuromeres forming the brain tube. Meek ('07) used the names 'prosomeres, mesomeres and rhombomeres' for those neuromeres that correspond to respectively prosencephalon, mesencephalon and rhombencephalon.

Von Kupffer ('06) in his contribution to Hertwig's Handbuch der Entwicklungsgeschichte, gave a survey of the already extensive literature on the existence of neuromeres up to that year. There was disagreement among the various authors of the time about whether or not it was possible to follow metameric phenomena from the neural plate stage to the closed neural tube stage and in this particular discussion Von Kupffer took a stand:

"Im Zeitpunkt wo sich das Hirnrohr rostral von der Epidermis ablöst (sind) im Gebiet des Vorder- und Mittelhirnes fünf, durch äussere Furchen geschiedenen Neuromeren zu sehen. Nimmt man dasjenige Furchenpaar, an welchem dorsal der Commissura Posterior zuerst sichtbar wird, als Grenzmarke, so fallen drei dieser Neuromeren auf das Vorderhirn, zwei auf das Mittelhirn. Alles weitere erscheint noch unsicher. So namentlich die Herleitung aller dieser Neuromeren einzeln für sich von ebensoviel primären Neuromeren der Neuralplatte resp. des massiven Neuralstrenges. Es empfielt sich daher, diese Neuromerie als sekundäre von der primäre zu unterscheiden. Die sekundäre Neuromerie des Hirnes verstreicht danach, ist überhaupt am Boden des Vorder- und Mittelhirngebietes nicht so ausgesprochen wie am Dache und an den Seitenwänden" (p. 166).

Thus he made a clearcut differentiation between primary and secondary neuromeres, primary neuromeres being called all those phenomena occurring before the closure of the neural tube, secondary neuromeres the segment-like vesicles of the closed neural tube. The three secondary prosencephalic neuromeres in question were called by him Telencephalon, Parencephalon and Synencephalon.

Although a number of interesting discussions on neuromerism has been

published in subsequent years (Haller, '29, '34; Streeter, '33; Kuhlenbeck, '35) it was the work of Bergquist ('52[a]), building on the results from the Swedish school (Palmgren, '21; Holmgren, '22, '25; Rendahl, '24) and on his own investigations on the diencephalon (Bergquist, '32) that has substantially furthered the acceptance of neuromerism as an essential feature of neurogenesis. In a fruitfull cooperation with Källén the principal similarity of development of the neural tube in all vertebrates was demonstrated (Bergquist and Källén, '54). In order to found a sound basis for the establishment of homologies they tried to reduce the complex adult nervous system to the more simple neuromeric units as morphogenetic entities.

The unsolved relationship between primary and secondary neuromerism which Von Kupffer recognized was solved by these authors who described a 'proneuromery' in the open neural tube (Källén and Lindskog, '53) which disappears after the closure of the tube, to give way to an interneuromeric phase I during which the neural tube shows no distinct neuromeric features. After this interneuromeric phase I (secondary) neuromeres appear and they are transformed *via* an interneuromeric phase II into (tertiary) postneuromeres. As both formation and disappearance of these phenomena should take place very quickly in a wave-like process sweeping along the neural tube, the verification of these facts is rather difficult.

The criteria used by Bergquist and Källén to delimit the proneuromeres and neuromeres apparently correspond tot Orr's description as quoted above. Postneuromeres are characterized by the existence of migration phenomena and consist of a number of migration areas from which the adult structures develop. As the neuromeres become indistinct when the migration areas develop the comparison between the areas and the neuromeres is very difficult. In the rostral part of the neural tube the configuration of the neuromeres is disturbed by several evagination processes and this adds to the difficulty mentioned above. Nevertheless Bergquist and Källén ('53[a], [b]) succeeded in observing a correspondence between neuromeres and postneuromeres. The criteria which a subdivision of a postneuromere should fulfill in order to be called 'migration area' could not be detected in the profuse literature on the subject. Vaage ('69), who recently made a thorough investigation on the segmentation of the primitive neural tube in the chick, could observe no reduction of neuromeres. Vaage denies the occurrence of interneuromeric phases; rather, he is of the opinion that during development a continuous increase of the number of neuromeres occurs by subdivision and transformation.

Neuromeres in the closed neural tube

The material studied in this investigation yielded no grounds for a sharp separation of neuromere phenomena into a primary and secondary series of

neuromeres. Since we are primarily interested in those phenomena that possibly persist, we will confine ourselves mainly to a discussion of neuromerism in the closed neural tube.

Within the prosencephalic area of the early neural tube Von Kupffer distinguished three segments, namely a telencephalon, a parencephalon and a synencephalon.

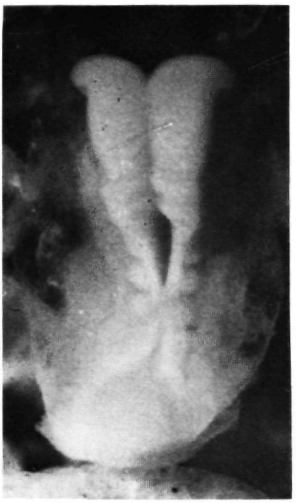
Rendahl ('24) in a thorough investigation of the development of the diencephalon of the chick distinguished within the parencephalon a parencephalon anterius and a parencephalon posterius. Within the synencephalon a precommissural and a commissural area could be observed by the same author. Bergquist and Källén ('54, '55) recognized four prosencephalic neuromeres, Neuromere I corresponding to the telencephalon, neuromere II being an optic neuromere, neuromere III the parencephalon (without the optic evagination) and neuromere IV the synencephalon. Vaage ('69) observed in the chick's prosencephalon a development from one single prosomere through a prosomere A and B into a further parcellation of no less than eight prosomeres, *i.e.*, two telencephalic (Pr 1, Pr 2), one optic (Pr 3), two parencephalic (Pr 5, Pr 6) and two synencephalic prosomeres (Pr 7, Pr 8), prosomere 4 being incorporated with the eventual prosomere 5.

In this chapter the number of prosencephalic neuromeres will be determined and described as it was observed in embryonic Chinese hamster brain of the stages E_{10}, E_{11}, E_{12} and E_{13}, sectioned in various directions.

OBSERVATIONS

Development prior to embryonic day 11

Prior to a discussion of the phenomenon of neuromerism it is necessary for two reasons to comment on the closing process of the anterior neuropore as observed in our material. First of all, the neuromeres are said to be oriented transversely to the brain axis, and the course of this brain axis is determined by the location of the anterior end of the brain. Secondly, the time of closure of the neural tube has been employed by Von Kupffer to separate early, primary neuromerism from later or secondary neuromerism. Empirically it was found, that at a postcoital age of ten days and twelve hours ($E_{10^1/_2}$) most embryos were starting fusion of the lateral margins of the neural plate at a level that is comparable to the cervical region of older stages. By this time the neural plate already exhibits a cranial flexure and the rest of the forming body is curled in a strong lordosis (fig. 6). Also the lateral margins of the neural tube on transversal section can be observed to be raised in its presumptive prosencephalic part even more than in its future rhombencephalic part (fig. 7).



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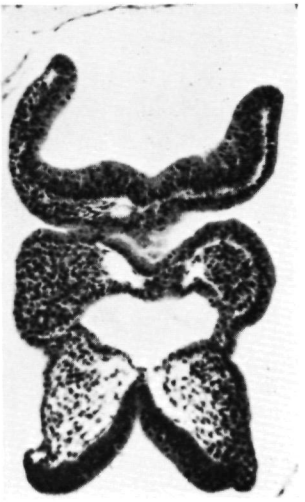

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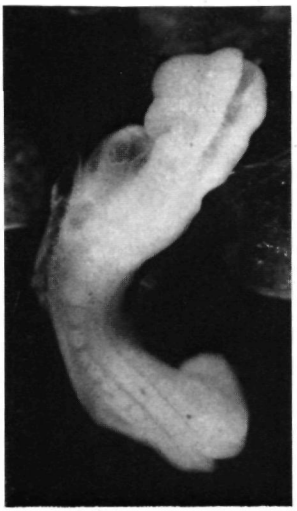

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Fig. 6 Photograph of the neural plate stage. The neural plate shows a general lordosis. The cephalic flexure is present in its rostral part. Closure has occurred in the cervical region ($E_{10}3/4$).

Fig. 7 Transverse section through the neural plate at the level of the cephalic flexure. Note the presence of a raised border in the prosencephalic division of the neural plate ($E_{10}1/2$).

Fig. 8 The formation of the brain tube; dorsal aspect of the embryo ($E_{10}3/4$).

Fig. 9 The formation of the brain tube; ventral aspect of the embryo. Note the presence of a slit-like anterior neuropore ($E_{10}3/4$).

When studying embryos at a slightly more advanced stage ($E_{10^3/4}$), it was observed that the fusion within the future cervical region of the deuterencephalon proceeds from the first place of contact in both a caudal and a rostral direction. Besides this a second region of fusion was observed in several instances within the archencephalon, rostro-dorsally to the cranial flexure. Thus an 'intermediate' neuroporus was formed between the two areas of fusion (fig. 8). In a few specimens the fusion in this second region was incomplete towards the rostral end of the neural plate, where a slit-like anterior neuropore could be seen (fig. 9).

At E_{11} all specimens studied showed a closed neural tube. The histologically sectioned series of recently closed neural tubes (several directions) were scrutinized for the presence of a neuroporic recess. In the basal part of the tube a distinct depression has been observed in the midline; it has been established that this recessus in later stages becomes the preoptic recess. However, we did not succeed in proving that this recess indeed coincides with the last point of closure of the anterior neuropore. Thus the course of the central brain axis in this study cannot be established by mere observation but rather depends on a definition of its anterior end. As has been discussed in chapter III we located the anterior end of the brain axis somewhere between the torus transversus and the velum transversum in the rostral wall of the brain tube.

In these early stages segment-like transverse bulges are present in the open neural plate; by definition these should correspond to the primary neuromeres of Von Kupffer ('06). When closed, the neural tube exhibits the circular neuromeres that were termed secondary neuromeres by the same author. Both these primary neuromeres and the early secondary neuromeres were termed proneuromeres by the Swedish school (Källén and Lindskog, '53) and represent the first set of neuromere-phenomena as observed by Bergquist and Källén ('54).

Neuromerism at embryonic day 11

On a superficial view the external aspect of the brain in the E_{11} embryo suggests the presence of three vesicles, connected by constrictions. In the textbooks these vesicles are called prosencephalon, mesencephalon and rhombencephalon. On closer scrutiny, however, several segment-like structures are observed within each of these 'vesicles', each possessing its own individual outline and configuration (fig. 10). These 'neuromeres' can be called prosomeres, mesomeres and rhombomeres (Meek, '07, '09) according to the 'vesicle' to which they belong. Each neuromere fulfills Orr's (1887) criteria: it is separated from its neighbours by an external dorsoventral constriction, and opposite this is an internal sharp dorsoventral ridge, and on section, each lateral half of a neuromere appears as a small arc of a circle.

The question can be asked how the neuromeres in this stage correspond to the scale of neuromerisms as discerned by different authors. According to Von

Kupffer's definition the E_{11} neuromeres can be designated as secondary neuromeres; in the Bergquist-Källén nomenclature they should be called proneuromeres.

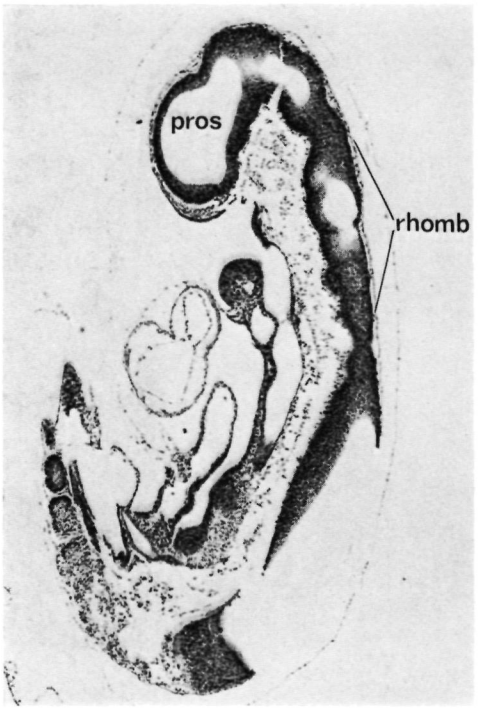


Fig. 10 Sagittal section of the brain tube in an E_{11} embryo.

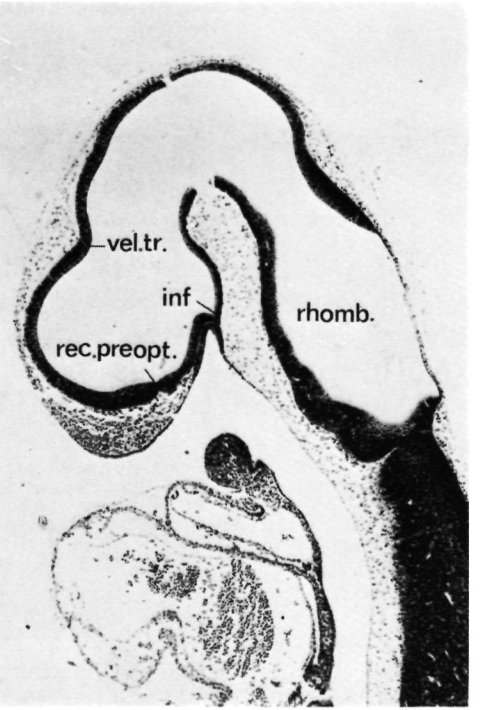


Fig. 11 Sagittal section of the brain in the E_{12} embryo.

As appeared from the review of literature presented above, neuromerism can be described as a morphogenetic process. It is, however, closely linked up with the histogenesis of the brain. In this chapter, the relationships between neuromerism and histogenesis are intentionally left out of consideration but they will be considered later on (chapter VI and VII).

In the E_{11} neural tube a mesencephalic neuromere presents itself lying on top of the plica encephali ventralis. It is bordered caudally and rostrally by external interneuromeric fissures. The part of the brain rostral to it is subdivided by circular constrictions which are seen on section as external fissures and internal eminences. Most rostrally a large prosomere is observed, delimited caudally by an interneuromeric eminence running from the velum transversum basalward to reach the midline just rostral to the infundibular anlage. Within this large subdivision of the brain tube two early evaginations become manifest, *i.e.,* the rostrodorsally directed outgrowth of the cerebral hemisphere and the laterally directed optic evagination.

The primitive infundibular bulge is seen caudal to this first prosomere. It forms the basal part of a second neuromere, the parencephalon. Between this parencephalon and the mesomere on top of the plica encephali ventralis, another neuromere is observed. In this instance it can not be decided whether a synencephalon is present or an additional mesomere. This is due to the absence of reliable landmarks in the neural tube's wall at this stage and to the fact that occasionally two neuromeric components are described within the future mesencephalon (Meek, '10; Palmgren, '21; Adelmann, '25).

Neuromerism at embryonic day 12

At the E_{12}-stage the proportions between the thickness of the wall of the neural tube and the ventricular lumen have changed, so that the neural epithelium appears thinner, but no absolute decrease of it in thickness has taken place (fig. 11).

Most rostrally the large prosomere shows a striking differentiation; in it the telencephalic evagination has increased considerably and the optic vesicle is

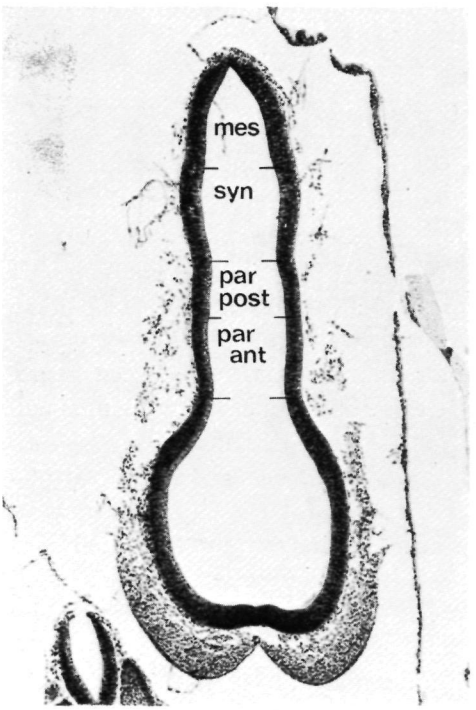


Fig. 12 Transverse section of the E_{12} embryo. The ventricular interneuromeric crests can be discerned.

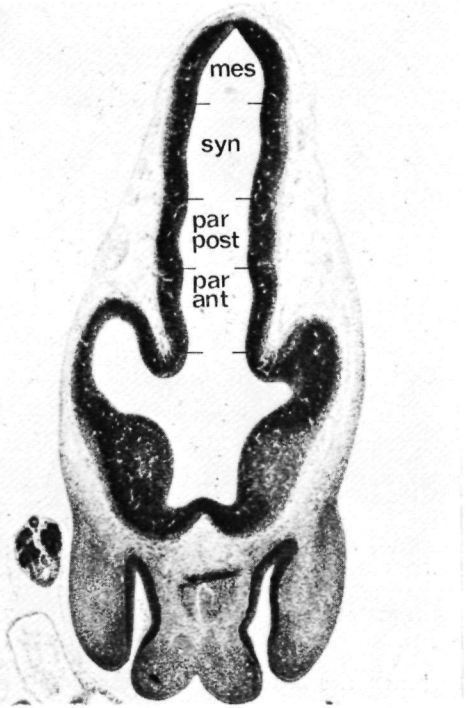


Fig. 13 Transverse section of the E_{13} embryo. At several places a mantle layer can be observed

separating from the prosencephalon but for its stalk; this optic evagination entails that most of the material from which the optic vesicle originates, is withdrawn from the median ventricle. This may explain the small size of the optic neuromere in later stages. Caudal to the latter a separate postoptic neuromere is observed, coinciding in its basal part with the postoptic recess and originating all the same from the primary large rostral prosomere. The latter thus gives origin to the telencephalic, the optic and the postoptic neuromeres. Within the parencephalon a subdivision has taken place by an interneuromeric constriction that resulted in the formation of a parencephalon anterius and a parencephalon posterius as two separate neuromeres (fig. 12).

In $E_{12}1/_2$ for the first time a synencephalon can be indicated with certainty; the specimen illustrated shows an (only weakly developed) anterior mesomere (fig. 14).

Neuromerism at embryonic day 13

In E_{13} the number of neuromeres has attained its maximum. The septa that are formed by the crowding of cells in the interneuromeric crests at some places show histologic characteristics indicative of differentiation. These herald the more permanent structures that will develop in the interneuromeric crests and that will permit us to follow the interneuromeric borders from these early stages throughout development (fig. 13).

The neuromeres of these stages can be called secondary neuromeres according to Von Kupffer's terminology and neuromeres according to the Bergquist and Källén school. In our material in between the proneuromere and neuromere stages in this sense no interneuromeric phase I could be observed, however, and the opinion is advanced that neuromeres originate by the simple subdivision of proneuromeres. Vaage ('69), who studied avian material, arrived at a similar conclusion.

The telencephalic and optic evaginations claim the greater part of the original large rostral neuromere (fig. 15). The border between the telencephalon and the remainder of the prosencephalon can be followed from the velum transversum on the external surface in a ventral direction as the sulcus telodiencephalicus. This sulcus passes rostrally to the optic stalk reaching the basis of the brain tube just rostral to the preoptic recess, where it is continuous with its fellow on the other side. The sulcus in question corresponds to the ventricular torus hemisphaericus that can be followed from the velum transversum with a rostrodorsal concavity around the incipient foramen of Monro to the torus transversus in the neural tube's terminal wall.

In these early stages this torus can be interpreted as indicating an interneuromeric constriction coinciding with the initial telodiencephalic boundary. During further development complex morphogenetic and histogenetic events

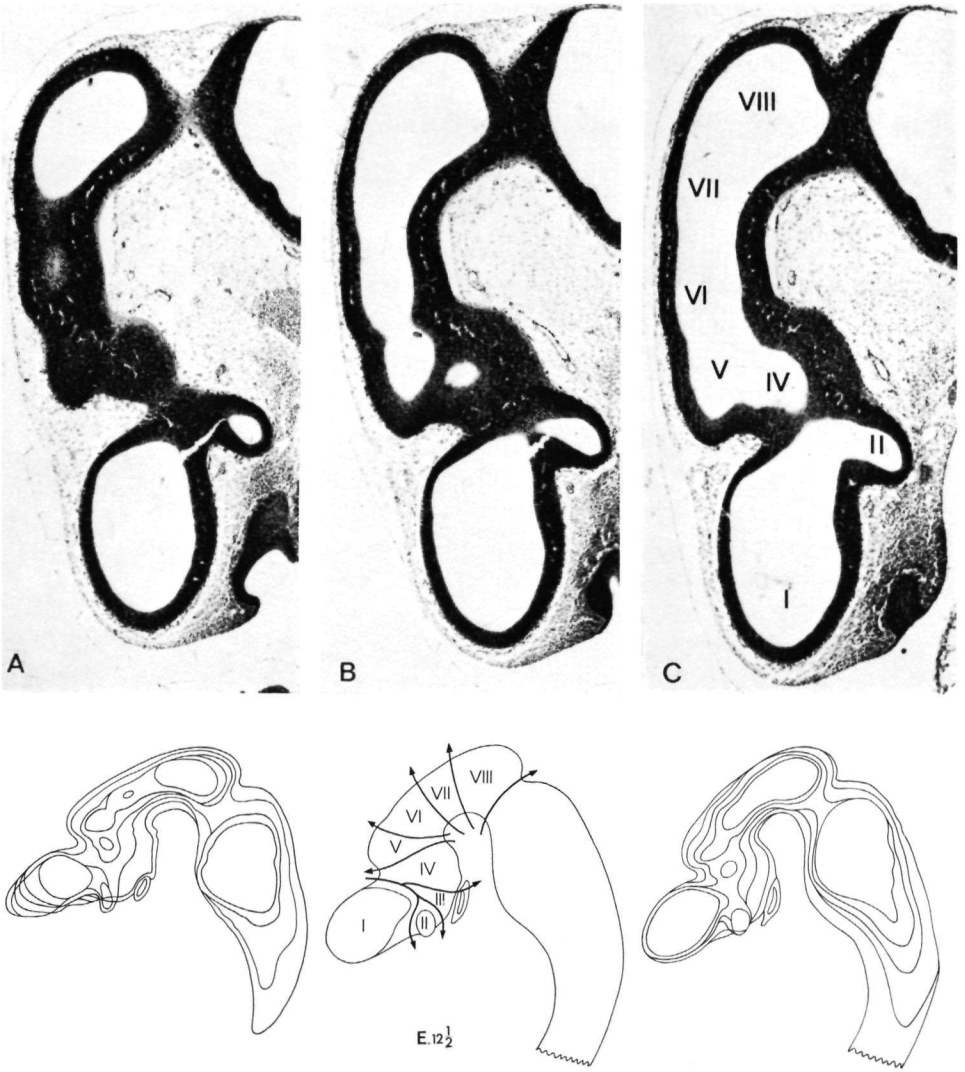


Fig. 14a. Sagittal sections of the $E_{12}\frac{1}{2}$ embryo. The Roman numerals indicate the neuromeres. Fig. 14b. The neuromeric pattern at $E_{12}\frac{1}{2}$ reconstructed from a sagittally sectioned series.

occur in this particular area which obscure this boundary. The great confusion concerning the boundary between the telencephalon and the diencephalon (for a discussion see *e.g.* Von Kupffer, '06; Johnston, '09; Kingsbury, '20, '22; Dart, '25; Källén, '51 and Vaage, '69) is related to the early disappearance of this landmark.

The optic neuromere can be thought of as withdrawing neurogenic material

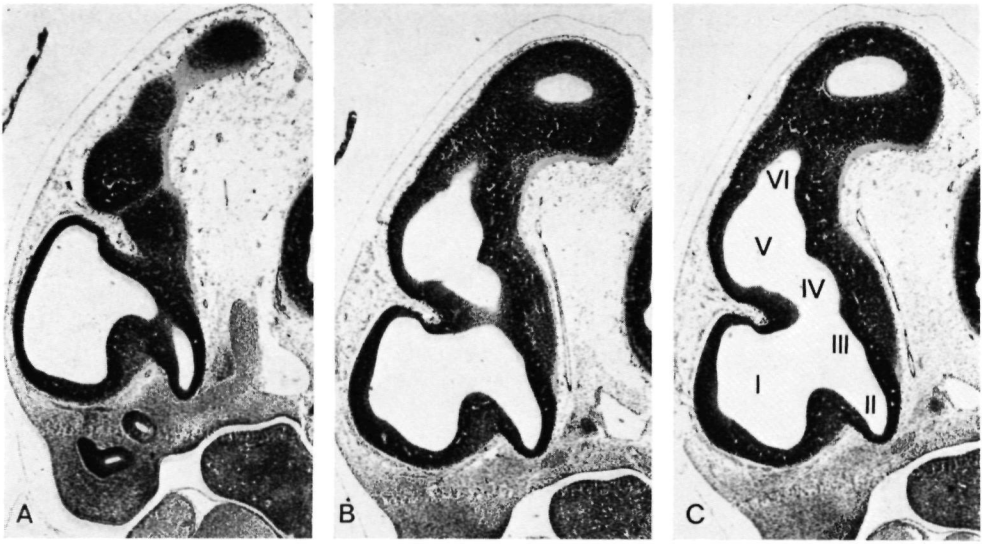


Fig. 15 Sagittal sections of the E_{13} embryo. The Roman numerals indicate the neuromeres.

from the prosencephalic wall. Essentially it should have formed a complete ring or segment of the neural tube; its dorsal representative, however, is not clear and possibly is a constituent of the diencephalic half of the torus hemisphaericus which will develop later on into the eminentia thalami. From there, the caudal border of this optic neuromere can be traced basally to reach the midline as a column of differentiated tissue dorsal to the primordial optic chiasm.

The postoptic neuromere described in E_{12} in front of the parencephalon is on the way of losing its identity in the basal regions. It is bound to form a regio postoptica together with the rostral region of the infundibular part of the parencephalon anterius (*vide infra*). In the parencephalon the parencephalon anterius has increased considerably when compared to the parencephalon posterius. The interparencephalic crest between the two has a direction from the tuberculum posterius basally to a point somewhat caudal to the velum transversum dorsally. It is quite prominent at this stage and it contains a differentiated core. Later on in this crest the zona limitans intrathalamica develops marking the place of separation between the pars dorsalis thalami and pars ventralis thalami. This neuromeric border thus remains recognizable throughout development. The parencephalon posterius is bordered caudally by a crista interneuromerica in which laterally the primordial fasciculus retroflexus can be seen. This fibre bundle will mark later on the caudal border of the pars dorsalis thalami with the synencephalon. Between the parencephalon posterius and the mesencephalic bulge a synencephalon is observed. It is bordered caudally by a crista interneuromerica that coincides with the caudalmost fascicles of the

commissura posterior. The neuromeric borders between parencephalon anterius, parencephalon posterius, synencephalon and mesencephalon loose their distinctness in the basal part of the brain.

Surveying further development of the neuromeres that are thus established in E_{13}, it can be stated that some interneuromeric borders can be traced all the way up to the adult brain, while others become obscured and perish in a pattern that, secondary to this neuromeric stage, is originated by histogenetic events in the neural tube's wall. By this process the neuromeres will develop into postneuromeres in Bergquist's sense (Bergquist and Källén, '55). This statement can be amplified with the help of the E_{14}-stage, in which this process has progressed to some extent (chapter VII).

DISCUSSION AND CONCLUSION

The location of the anterior neuropore

A necessary prerequisite for the study of neuromerism was an investigation into the existence of an anterior neuropore *s.s.*. This structure is employed in literature in order to locate the anterior ending of the central brain axis and therefore forms an important landmark; besides, the time of its closure was used by Von Kupffer as a criterion to distinguish between primary and secondary neuromerism.

As regards the way in which the formation of the neural tube takes place, no confirmation of the classical theory on this subject could be obtained. The first place of fusion is located in the future cervical region. Thus a large neuropore is originated and this becomes subdivided by a second fusion of the tube, just rostral to the cephalic flexure. In this way an intermediate neuropore is formed in the future rhombencephalic area and an anterior neuropore *s.s.* that is located between the chiasmatic anlage (the rostralmost part of the neural plate) and the second region of fusion. The exact way of closure of the anterior neuropore *s.s.* could not be detected. The data from literature on the location of the recessus neuroporicus as a remnant of this anterior neuropore, are rather vague (*cf.* Bartelmez and Evans, '26; Sternberg, '27), but tradition places it in the dorsalmost part of the stretch of epithelium between chiasma opticum and velum transversum. No vestige of it, however, could be found in the primordial lamina terminalis (or dorsal to it) in our series. The only recess that could be observed in the rostral wall of the neural tube was, without any doubt, traceable into the future recessus preopticus, and no separate neuroporic recess could be discerned. Therefore, it was decided to define the anterior ending of the central brain axis in the midline, located somewhere between the velum transversum and the torus transversus (see also chapter III).

The study of neuromerism phenomena in the neural tube during development with the use of an extensive embryological material from one species, gives an opportunity to take a stand on a number of questions at issue in the literature on this subject. We are in a position to confirm the existence of a segmented appearance of the curved neural plate even before closure of the neural tube starts. The structures responsible for this configuration persist after the closure of the tube and they then meet the criteria drawn up for neuromeres by Orr in 1887. No correspondence, however, exists between the number of the structures present before the start of the closing process and the number of neuromeres in the early closed neural tube. An explanation of this fact probably has to be looked for in the causes underlying the formation of neuromeres. It has been shown by Bergquist ('32), Rudebeck ('45) and Källén (51, '52[a], '56), that these neuromeric bulges coincide with proliferation maxima in the developing neuroepithelium. They believe that the appearance of the former is caused by the development of the latter. The increased number of neuroepithelial cells produced by the ventricularly located mitotic activity, causes a bulging outward of these particular parts of the neural tube's wall. They alternate with regions that proliferate less profusely, and this could explain the appearance of 'constriction'-rings. The spatial distribution of the proliferation maxima, however, is not constant, but varies according to the stage of development, its number increasing with age. In this study, no counts of mitoses were performed. Our observations on the neuromeres did not show any contradiction with the explanation of the causes of neuromerism as proposed by Källén.

The distinction between a primary and a secondary neuromerism, respectively before and after the closure of the anterior neuropore, as made by Von Kupffer ('06) and Kuhlenbeck ('35) may be traced back to the observation of a different spatial distribution of proliferation maxima in the neuroepithelium. The value of this distinction, therefore, can be seriously questioned.

The neuromeric structures present in the neural tube at the moment of its closure are called proneuromeres by Källén and Lindskog ('53). Through an intermediate, interneuromeric phase, in which the proneuromeres vanish, a second generation of neuromeric structures, the neuromeres, originate (Bergquist, '52; Bergquist and Källén, '54). The formation of proneuromeres by proliferation maxima, their disappearance by the slowing down of mitotic activity and the generation of neuromeres by proliferation maxima of the second order, show a temporo-spatial relationship that makes the three phenomena comparable with waves sweeping along the neural tube in a specific direction. As the alterations take place at a rather fast speed, both proneuromeres, interneuromeric phase and neuromeres can be observed in one single specimen at different places along the neural tube, and very closely spaced series are necessary in order to

confirm the mechanism of the formation of neuromeres as proposed by the Swedish authors. We were not able to either confirm or to deny their conclusions on this subject.

We concluded that a progressive subdivision of early neuromeres exists, probably caused by the alternating presence of proliferation maxima, resulting in the formation of a definitive generation of neuromeres. By force of the differentiation of neural tissue in the interneuromeric borders of that stage, this definitive generation of neuromeres forms the starting point for the eventual transformation of the neuro-epithelial brain tube into the embryonic brain of later stages. In our material of 13 days postcoital age the definitively established prosencephalic neuromeres can be described in summary as follows (see also fig. 16): A synencephalon is distinguished rostral to the mesencephalon, separated from the latter and from the parencephalon in front of it by circular external fissures. These fissures correspond to ventricular eminences, that are demonstrable most clearly in the dorsal half of the neural tube.

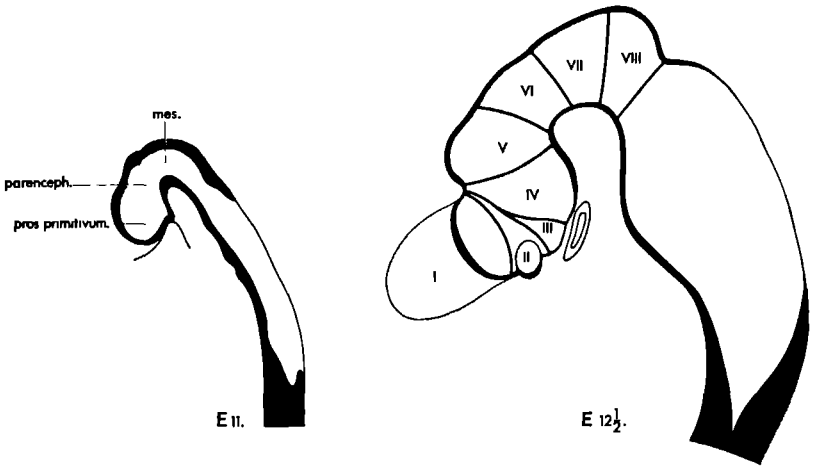


Fig. 16a. Schematic representation of the diencephalic neuromeres in the E_{11} Chinese hamster. Fig. 16b. Schematic representation of the diencephalic neuromeres in the $E_{12}\frac{1}{2}$ Chinese hamster (cf. text).

The parencephalon is bounded rostrally by a similar constriction that runs from the velum transversum towards the basal parts, crossing the midline just rostral to the infundibular stalk. The parencephalon is divided into a parencephalon posterius and a parencephalon anterius by an interneuromeric constriction running from the roof plate, just caudal to the velum transversum, towards the base of the neural tube and crossing in the mamillary region.

Rostral to the parencephalon, originally a single neuromere is present. This structure was labeled prosencephalon primitivum by Mihalkovicz (1877) and

Prosomere A by Vaage ('69). From its lateral wall the optic vesicle evaginates, thus withdrawing neurogenetic tissue from the neural tube's wall. This optic anlage is considered part of a separate neuromere by several authors (for a discussion see Bergquist, '52). From the original 'prosencephalon primitivum' in the E_{13}-stage have developed a prosencephalon secundarium (or telencephalon) most rostrally, an optic neuromere and a postoptic neuromere.

The postoptic neuromere is limited caudally by the rostralmost parencephalic border (already fading away at this stage); rostrally it borders on the optic neuromere by an interneuromeric crest reaching from the velum transversum dorsally towards the middle of the chiasmatic plate basally.

The rostral border of the optic neuromere, that is at the same time the caudal limit of the prosencephalon secundarium or telencephalon, is formed by the torus haemisphaericus which curves from the velum transversum around the caudo-basal border of the primitive foramen of Monro towards the torus transversus which crosses the midline. It is the early disappearance of this important landmark, becoming obscured during later stages by complex morphogenetic and histogenetic events, that has caused considerable confusion in literature as to the proper understanding of the telodiencephalic boundary. This question will be discussed to some extent in chapter VII, on mantle layer development and in chapter IX.

The significance of the set of neuromeres that thus has established a starting point for the further development of the nervous system and the relationship between the diencephalic neuromeres and other developmental phenomena also will form the subject of discussion later on in this study.

CHAPTER V

MORPHOGENESIS OF THE VENTRICULAR SURFACE

INTRODUCTION

In the preceding chapter neuromeres were introduced as distinct structures within the neural tube. They possess a number of characteristics, including the bulging outward of the neuromere's central part and the existence of circular interneuromeric constrictions. At the ventricular side of the neural tube the interneuromeric constrictions show up as eminences, separating the neuromeric cavities. This aspect of neuromerism, dealing with the ventricular relief of the early central nervous system raises the question of the possible relationship between this neuromere-caused relief and the sulci which can be observed on the ventricular surface during later stages of development. As both neuromeres and ventricular sulci are used by different authors as criteria of subdivision of the brain, a study of the relations between the two phenomena seems warranted.

The sulci that have been generally recognized within the mammalian embryonic diencephalon of later stages are: a sulcus diencephalicus ventralis, separating the hypothalamus from the pars ventralis thalami; a sulcus diencephalicus medius, separating pars ventralis thalami and pars dorsalis thalami; and a sulcus diencephalicus dorsalis, between pars dorsalis thalami and epithalamus. Apart from the three diencephalic sulci just mentioned two more sulci are discerned within the hypothalamic area; rostrally a sulcus intraencephalicus anterior runs from the foramen of Monro to the original optic evagination and caudally a sulcus lateralis infundibuli is branching off from the sulcus diencephalicus ventralis in the direction of the infundibular stalk.

The morphological position of the diencephalic sulci has been the object of much debate. According to His (1892, 1895) and Herrick ('10, '17) a longitudinal or horizontal position had to be attributed to the diencephalic subdivisions that were discerned by them. The diencephalic sulci in between were also considered to run longitudinally. The longitudinal subdivision of the lateral wall of the neural tube into an alar and a basal plate, separated by a sulcus limitans, as proposed by His (1888) and worked out by Gaskell (1889), Strong (1895), Herrick (1899) and Johnston ('02), extends according to these authors beyond the deuterencephalon into the archencephalic derivatives. Thus the sulcus diencephalicus ventralis was considered the foremost part of the sulcus limitans that, according to His (1888), Johnston ('09) and Streeter ('11), ends in the recessus preopticus.

Schulte and Tilney ('15) and Kingsbury ('20, '22) on the other hand arrived at the conclusion that the sulcus limitans arches rostrobasally and terminates in the recessus mamillaris. Kuhlenbeck ('29[b]) agreed with Kingsbury as far as the end of the sulcus limitans is concerned, but he otherwise stuck to a longitudinal position of the diencephalic sulci: "Das Herrickssche Zonensystem liegt also als neues System gewissermassen in der rostralen Verlängerung des Hisschen Zonensystems bzw. steht senkrecht auf dem Ende des Sulcus limitans infolge der dorsalkonvexen Krümmung dieses letzteren Furche" (o.c. p. 55).

In contrast to the authors mentioned thus far, Haller ('29) in a study on the development of the diencephalon and mesencephalon in various groups of vertebrates, concluded that the three cardinal diencephalic sulci take a basically transverse course and can be related directly to the preceding neuromeres; no grounds could be detected by him for a subdivision of the diencephalon into longitudinal zones. These results were confirmed in the extensive study by Bergquist ('32) on the development of the diencephalon in lower vertebrates, which he later extended to some groups of higher vertebrates as well (Bergquist, '52[a, b, c], '53[a, b], '54[a, b]).

The sulcus limitans mentioned above, is considered a most important landmark in the brain. It separates two regions, namely the basal plate and the alar plate, the former containing the motor centres, the latter the sensory centres. Therefore, this sulcus possesses not only a morphological, but also a functional significance. Hence its position is a much debated one. Historically the sulcus limitans was identified with the sulcus diencephalicus ventralis by His (1888), Von Kupffer ('06), Johnston ('09), Streeter ('11), Bok ('26), Spatz ('27) and Winkler ('33). As far as its rostral ending is concerned three opinions prevail:

1) His (1890), Johnston ('09) and Streeter ('11) were able to follow it into the recessus preopticus.

2) According to Bok ('26), Spatz ('27) and Winkler ('33) the sulcus in question passes through the foramen Monroi into the lateral ventricle, where it becomes continuous with the sulcus terminalis.

3) A number of authors, however, concluded an absence of a sulcus limitans within the rostral part of the prosencephalon. Schulte and Tilney ('15) and Kingsbury ('20, '22) decided that it ends with a rostrodorsal convexity in the mamillary region.

The preceding survey of the literature shows that there is a great deal of confusion with respect to the sculpturing of the ventricular surface of the diencephalon. In this chapter the development of the diencephalic ventricular sulci will be analysed and their morphological position will be evaluated. In this context an attempt will be made to find an answer to the following questions:

1. What is the fate of the neuromeric depressions during further development?
2. What is the morphological nature of the three sulci which subdivide the diencephalon into the well-known four stories?

3. Does the sulcus limitans of His extend into the diencephalon and if so, where does it end?

When studying the ventricular relief of the brain during development one has to be aware of the fact that this relief is continuously changing as an expression of the histogenetic processes that occur in the wall of the neural tube. The temporo-spatial patterning, inherent to histogenesis, will be studied later on in this study. It has to be kept in mind that an understanding of the meaning of the ventricular relief cannot be obtained without a profound knowledge of this patterned process.

OBSERVATIONS

For the analysis of the shape and sulcal pattern of the ventricular surface a large number of graphical reconstructions has been employed. A selection of them is shown in this chapter. A drawback of the technique of graphic reconstruction, that is never mentioned in the literature, is its susceptibility to the direction of sectioning of the brain that is studied. As differences in level of the ventricular surface are plotted on the midsagittal plane, only those prominences and excavations are in an optimal position for observation, that are oriented at right angles to the plane of sectioning. It is important to have this in mind when considering the figures obtained by the technique mentioned. The reliability of the description gains by the study of several specimens of the same developmental stage, sectioned at different planes.

Ventricular aspect at embryonic day 12

The relief shown by the diencephalic wall in this stage consists of a number of rather shallow excavations that gradually merge into one another. An exception is the optic stalk that is rather deep and that widens medially into the optic stalk conus and thus is attached to the lateral wall of the IIIrd ventricle. Fig. 17 shows several features that have been discussed in earlier chapters. The evaginating hemisphere accentuates the velum transversum and shows a very broad communication of the lateral ventricle with the third ventricle. The optic stalk is still open and its lumen communicates with the cavity in the optic vesicle. A ventricular ridge passes from the velum transverum basally to the region immediately rostral to the optic stalk. This ridge is the torus hemisphaericus.

As has been pointed out in the previous chapter, the torus hemisphaericus is considered to represent an interneuromeric boundary. Directly caudal to this boundary the optic neuromere is situated. The caudal limit of this optic neuromere is formed in this stage by a prominence running from a point in the basal midline, located behind the optic stalk and in front of the infundibulum, towards the dorsal velum transversum.

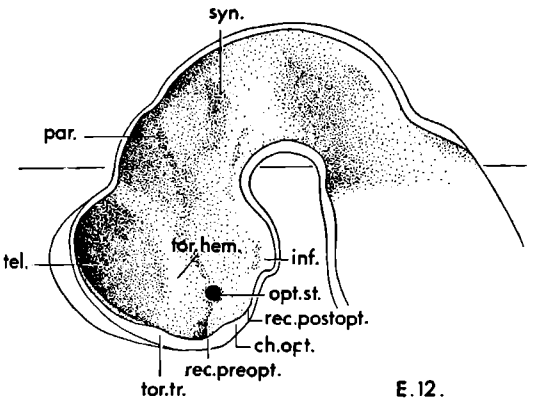


Fig. 17. Reconstruction of the ventricular surface in the E_{12} Chinese hamster.

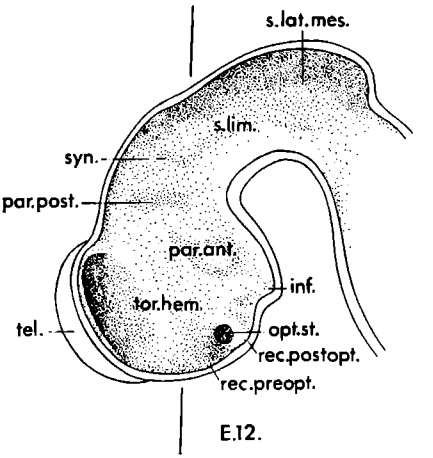


Fig. 18 Reconstruction of the ventricular surface in the E_{12} Chinese hamster.

Other neuromeres that can be discerned more caudally in the brain tube are, in a rostrocaudal sequence, the parencephalon, the synencephalon and a mesencephalic neuromere. The parencephalon reaches up to the level of the velum transversum. In some reconstructions of the 12th day (fig. 18) it already shows a subdivision by a crest running between velum transversum and tuberculum posterius, *i.e.*, the eminentia interparencephalica. This subdivision results in the formation of a parencephalon posterius, the future pars dorsalis thalami, and the parencephalon anterius, which eventually gives origin to the pars ventralis thalami and the infundibulum. More caudally an external synencephalic bulge can be observed (fig. 19) corresponding to an internal groove or excavation as illustrated by fig. 18.

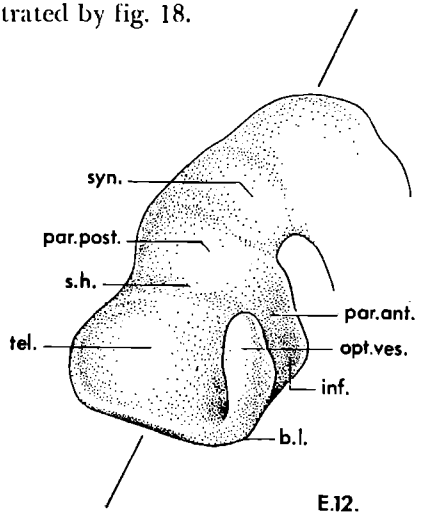


Fig. 19 Reconstruction of the external surface of the brain tube in the E_{12} Chinese hamster.

Ventricular aspect at embryonic day 13

During the thirteenth day of development the neuromeres are still easily recognized as has been pointed out in the preceding part of this study. Figure 20 therefore shows many features that have already been discussed in chapter III and IV. The midline changes which have taken place consist of the appearance of the epiphyseal anlage and of the altering of position of those parts that are separated by the recessus postopticus and by the infundibulum On the ventricular surface the bulging outward of a synencephalon, a parencephalon posterius and a parencephalon anterius are marked (fig. 22).

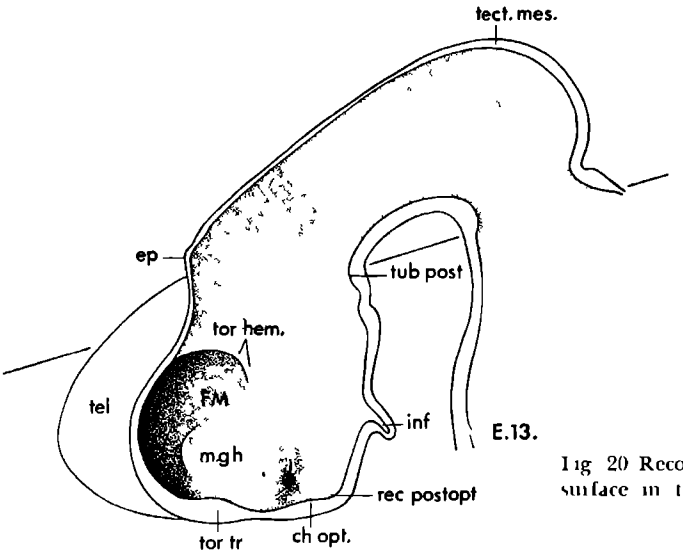


Fig 20 Reconstruction of the ventricular surface in the E_{13} Chinese hamster

Within the caudobasal half of the torus hemisphaericus a medial ganglionic hill or medial striatal eminence is observed (fig. 20). The outgrowth of this structure obscures the position of the torus hemisphaericus and therewith of the original telodiencephalic boundary as defined by Mihalkovicz (1877), Meek ('07, '09, '10) and Kuhlenbeck ('54, '56) (figs. 20, 21, 22). The caudal limit of the medial ganglionic eminence becomes accentuated by a groove which is situated somewhat in front of the conus of the optic stalk (fig 20) This sulcus may be called the sulcus intraencephalicus anterior primitivus. The foramen of Monro is narrowed by the changes taking place (fig. 21, fig 22).

In appropriately sectioned series it can be further observed that the groove formed by the points of largest diameter of the neural tube (in its diencephalic and mesencephalic parts) can also be followed into the optic stalk This groove can be traced into the sulcus diencephalicus ventralis of later stages Both sulcus intraencephalicus anterior and sulcus diencephalicus ventralis cross the interneuromeric borders rostral and caudal to the optic neuromere

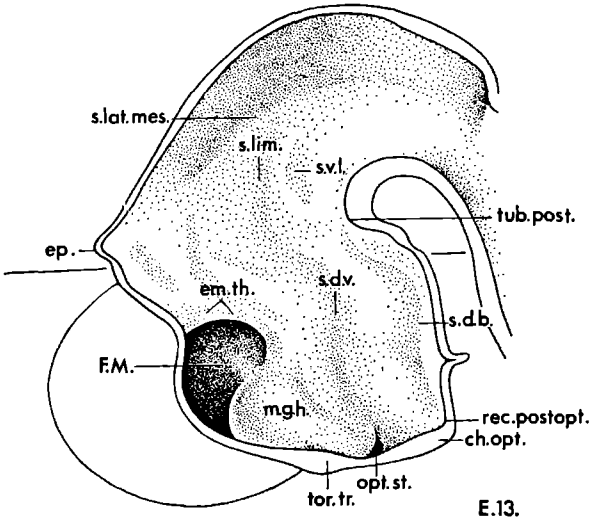


Fig. 21 Reconstruction of the ventricular surface in the E_{13} Chinese hamster.

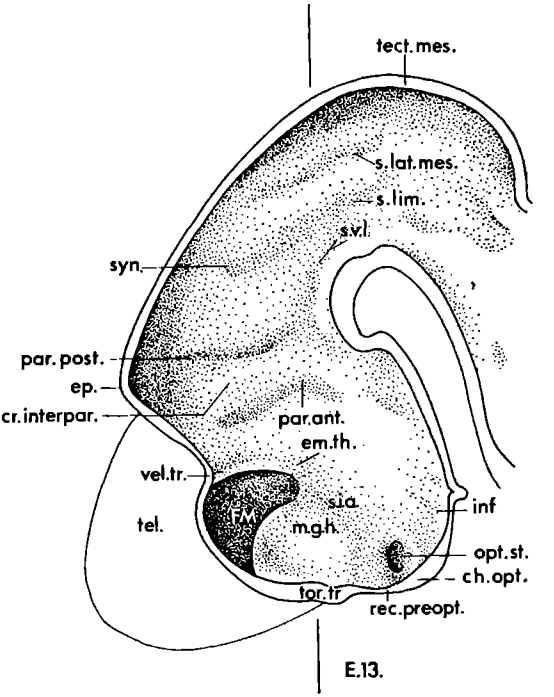


Fig. 22 Reconstruction of the ventricular surface in the E_{13} Chinese hamster.

During the 14th day the medial ganglionic eminence gradually grows beyond the dorsal margins of the foramen Monroi, thus changing the foramen into a relatively narrow slit (fig. 23, fig. 24). This fact most probably is of a multiple origin. The ballooning of the growing hemisphere undoubtedly causes a bending basalward of the basolateral parts and so of the telodiencephalic border zone in which the medial striatal eminence develops. The preoptic region and medial ganglionic hill furthermore possess actively proliferating matrices. The former is the basis of the striatal eminence. The enlargement of this region plays a part in the transformations occurring in the telodiencephalic border zone

Ventricularly, three sulci were observed in this area at the E_{13}-stage; (1) the sulcus, marking the caudal boundary of the medial ganglionic hill which is in this stage a direct continuation of the sulcus terminalis; (2) the most rostral part of the sulcus diencephalicus ventralis which terminates in the conus of the optic stalk; and (3) a sulcus which begins in the conus just mentioned and which passes from there toward the incipient preoptic recess to meet its fellow of the opposite side; the latter sulcus is known as the sulcus opticus. At the E_{14}-stage, due to the expansive growth of the medial ganglionic hill, the sulcus mentioned under (1) is pushed caudalward and finally fuses with the sulcus mentioned under (2) (see figs. 24 and 25). This single sulcus from double origin is called the definitive sulcus intraencephalicus anterior. The third sulcus, *i.e.*, the sulcus opticus, gradually disappears The reason that we mention the presence of this transient sulcus is the fact that a number of authors confused this sulcus opticus with the sulcus intraencephalicus anterior (see *e.g.* Grönberg, '01, and J. E. Rose, '42).

So at this E_{14}-stage three sulci, namely the secondary sulcus intraencephalicus anterior, the sulcus terminalis and the sulcus diencephalicus ventralis meet in one point as is illustrated in the figures 23 and 24.

In the hypothalamic area a sulcus lateralis infundibuli (sulcus in hypothalamo) can be observed as a rudiment of the earlier infundibular neuromeric bulge (fig. 23).

A sulcus diencephalicus basalis (Luyendijk, '44) is observed in the basalmost part of the diencephalon. It marks the basal boundary between the hypothalamic cell cord, a longitudinally oriented area of early maturation which is extending from the tuberculum posterius to the optic chiasm, and the basalmost part of the hypothalamic anlage which remains thin walled.

In the region of the parencephalic sinuations no regular pattern can be observed. The wall of the neural tube is increasing in thickness here and only very slight differences in the ventricular surface are to be observed. The limit between the parencephalon anterius and posterius can be recognized, however, in microscopical sections. At the site of this boundary the zona limitans intra-

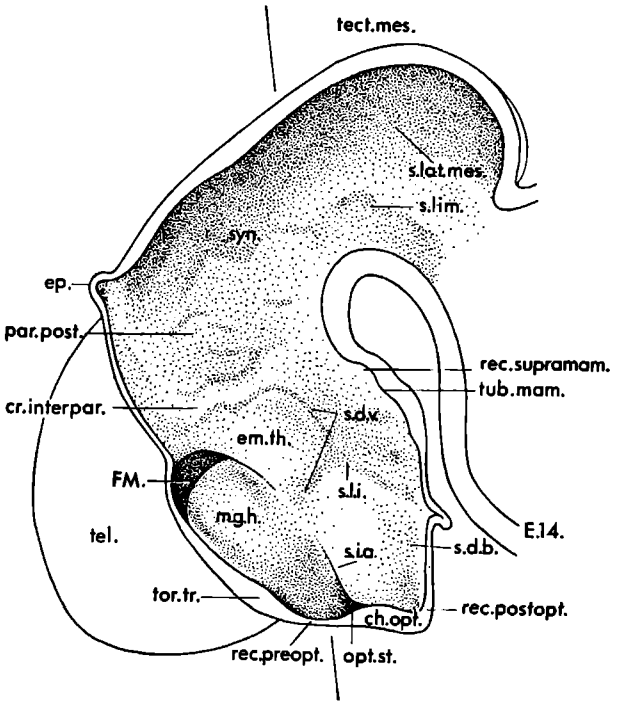


Fig. 23 Reconstruction of the ventricular surface in the E_{14} Chinese hamster.

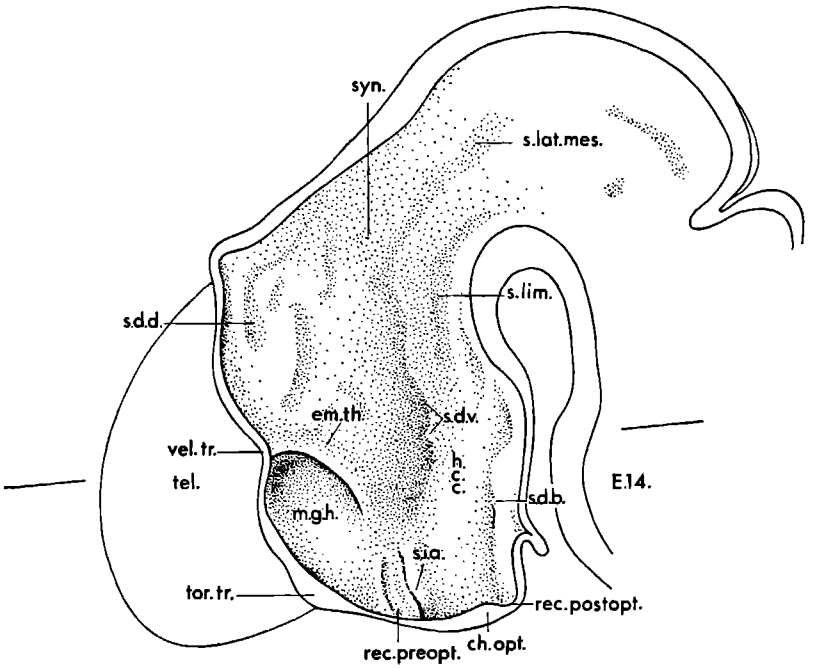


Fig. 24 Reconstruction of the ventricular surface in the E_{14} Chinese hamster.

thalamica is developing, a fibre layer which will develop into the lamina medullaris externa This interparencephalic crest is indicated in the reconstructions (fig. 23). The sulcus lateralis infundibuli which is located rostral to this crest can be considered a remnant of the parencephalon anterius neuromeric cavity.

Another sinuatio neuromerica which remains clearly visible is the recessus synencephalicus: this cavity gives origin to the recessus metathalamicus (fig. 23). At the stage under discussion the sulcus lateralis mesencephali seems to pass into this recess. The sulcus diencephalicus ventralis originates at the rostral end of the synencephalic recess and curves with a basally directed convexity in the direction of the foramen of Monro (see figs 24 and 25).

In the mesencephalic area two longitudinal sulci can be discerned: the sulcus limitans and the more dorsolaterally situated sulcus lateralis mesencephali. The former fades away in the region in front of the tuberculum posterius (fig. 25).

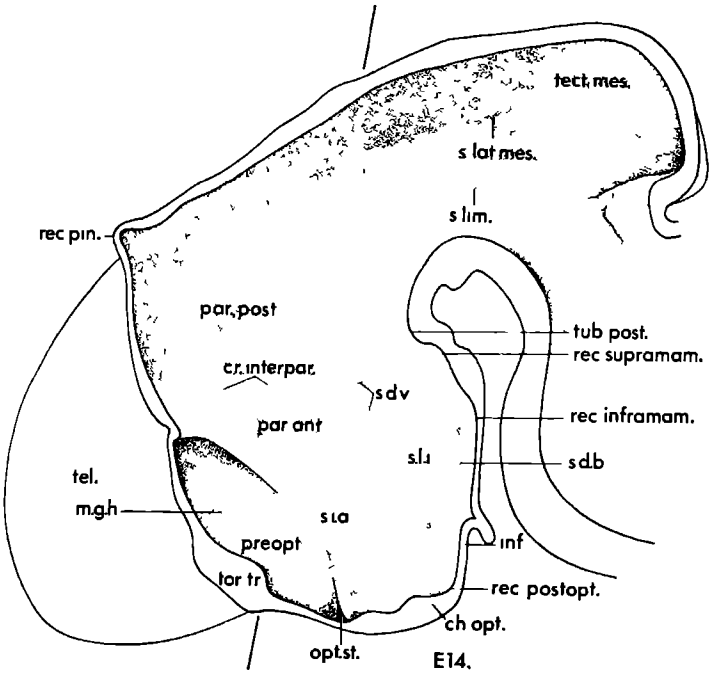


Fig 25 Reconstruction of the ventricular surface in the E_{14} Chinese hamster

Ventricular aspect at embryonic day 15

At this stage the relative preponderance of the pars dorsalis thalami and the increase of the surface of those regions that are bordering on the sulcus intraencephalicus anterior are the dominating features (fig. 26).

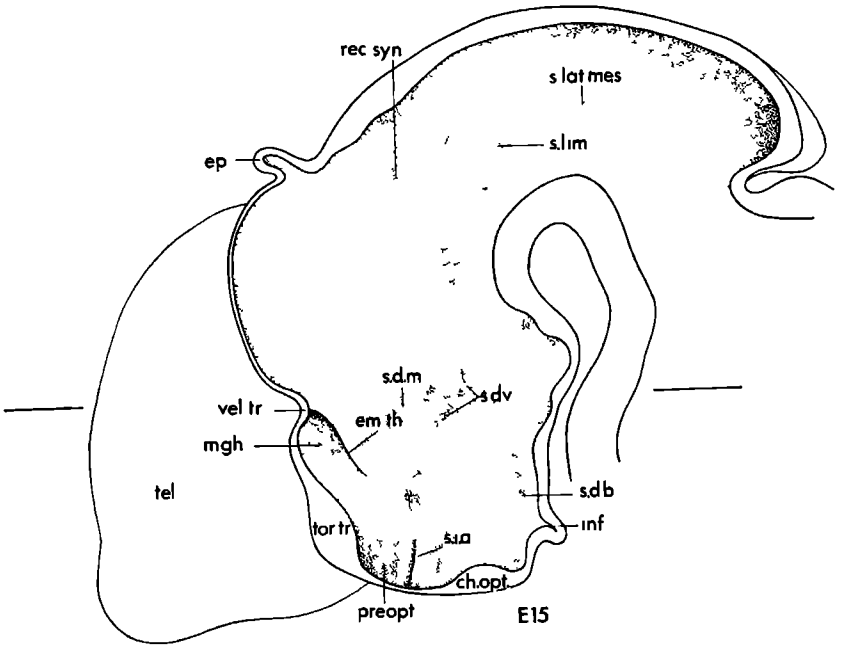


Fig 26 Reconstruction of the ventricular surface in the E_{15} Chinese hamster

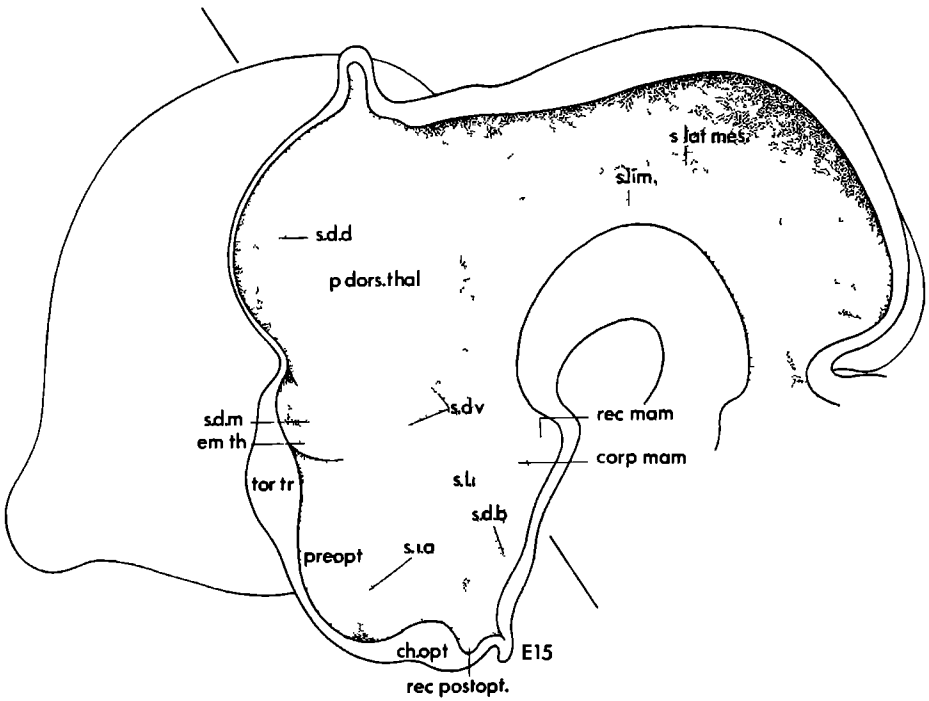


Fig 27 Reconstruction of the ventricular surface in the E_{15} Chinese hamster

The increase in thickness of the lateral wall of the parencephalon posterius as described in the preceding stage by now has effected the disappearance of the parencephalon posterius cavity and, instead, results in the prominence of the same area into the ventricular lumen. The phenomenon in question can be observed most readily in fig. 27. This bulging inward is attended by the appearance of sulci delimiting the dorsal part of the parencephalon posterius, *i.e.*, the future pars dorsalis thalami. These sulci are: the sulcus diencephalicus dorsalis and the sulcus diencephalicus medius. The latter in its caudalmost part joins the sulcus diencephalicus ventralis, s. hypothalamicus.

The basis of the medial ganglionic hill, extending from the basal end of the eminentia thalami to the torus transversus, was observed in earlier stages to consist of the preoptic region, which is located rostrally to the sulcus intraencephalicus anterior. In this E_{15}-stage both this region and the area just caudal to the sulcus intraencephalicus anterior (*i.e.*, the regio supraoptica) show a relative enlargement. Thereby the medial ganglionic hill seems to be displaced in a rostrodorsal direction (fig. 26, fig. 27).

The constellation of sulci around the foramen Monroi and the optic stalk has not been changed very much by these events.

The sulcus diencephalicus ventralis has become more pronounced by the increase in volume of the pars dorsalis thalami and of the tegmental areas. Caudally this sulcus retains a connection with the recessus synencephali s. metathalamicus. Rostrally it opens into an area where three sulci meet: the sulcus terminalis (between medial ganglionic hill and eminentia thalami), the sulcus intraencephalicus anterior and the sulcus diencephalicus ventralis.

The continuous proliferation of the so-called hypothalamic cell cord (Gilbert, '35) effects a reduction of the sulcus lateralis infundibuli. Since the most basal part of the hypothalamus now increases in thickness the sulcus diencephalicus basalis gradually becomes transformed into a shallow depression.

When studying the reconstructions it is rather difficult to trace the remnants of the parencephalic sinuations. The sulcus lateralis infundibuli represents the basal half of the parencephalon anterius. It is doubtfull whether the sulcus diencephalicus medius can be interpreted as the reduced dorsal half. The ventricular depression of the parencephalon posterius is greatly reduced. It is represented by the most rostral part of the sulcus diencephalicus dorsalis. Caudally the sulcus in question can be traced towards the recessus synencephali (s. metathalamicus), thus passing the interneuromeric boundary.

Ventricular aspect at embryonic day 16

The ventricular width decreases steadily by the increase in thickness of the wall of the neural tube and the ventricular relief becomes less pronounced (fig. 28).

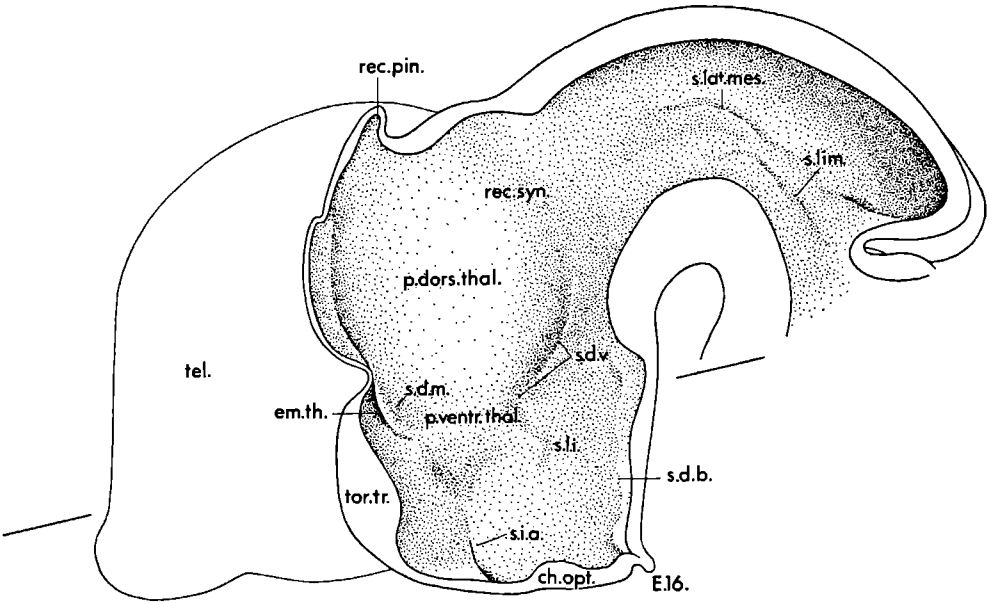


Fig. 28 Reconstruction of the ventricular surface in the E_{16} Chinese hamster.

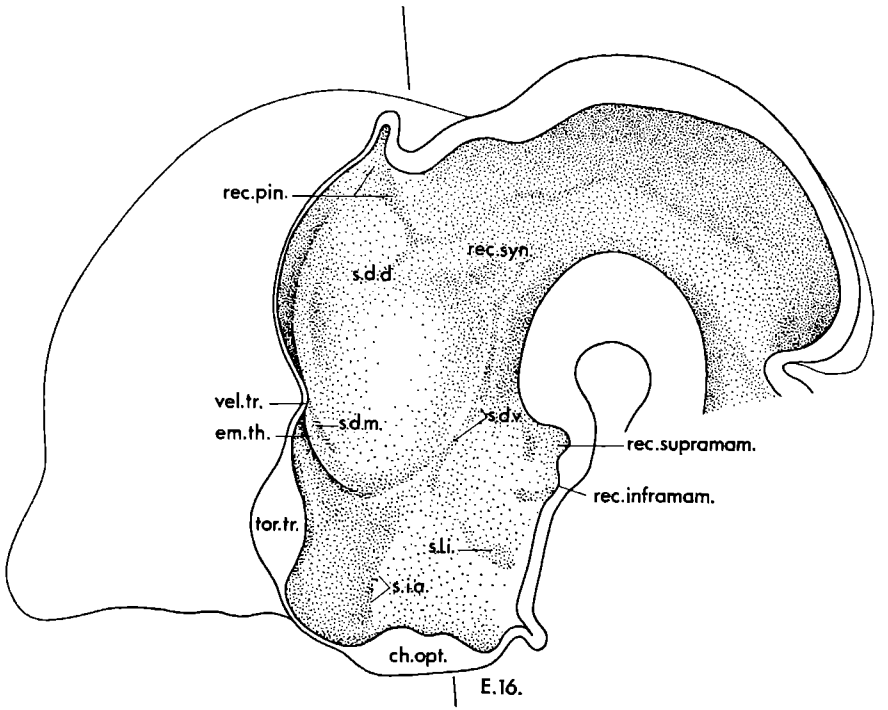


Fig. 29 Reconstruction of the ventricular surface in the E_{16} Chinese hamster.

A striking feature at this stage of development is the pars dorsalis thalami that has augmented its surface disproportionately when compared to the other diencephalic areas. Thereby the sulcus diencephalicus ventralis is accentuated and the foramen Monroi is narrowed to a slit. The sulcus intraencephalicus anterior by now is far less conspicuous and this probably is the reason that in the elder stages most authors consider the rostral continuation of the sulcus diencephalicus ventralis as running into the foramen Monroi (fig. 29).

A sulcus diencephalicus medius could not be observed clearly, although a slight indication of it was discerned just caudal to the foramen Monroi (fig. 29).

In the basal half of the diencephalon relations are altered by the increase of the regio supraoptica and of the pretegmental tuberculum posterius region. These changes result in the transformations discussed in chapter III.

In the border between the diencephalon and the mesencephalon the recessus synencephali forms a wide but shallow depression in which two diencephalic sulci, *i.e.*, the sulcus diencephalicus dorsalis and the sulcus diencephalicus ventralis, end. Since the longitudinal mesencephalic sulci also terminate in this depression it is hard if not impossible to judge whether the mesencephalic and diencephalic sulci are continuous.

In fig. 29 the communication between the sulcus diencephalicus ventralis and

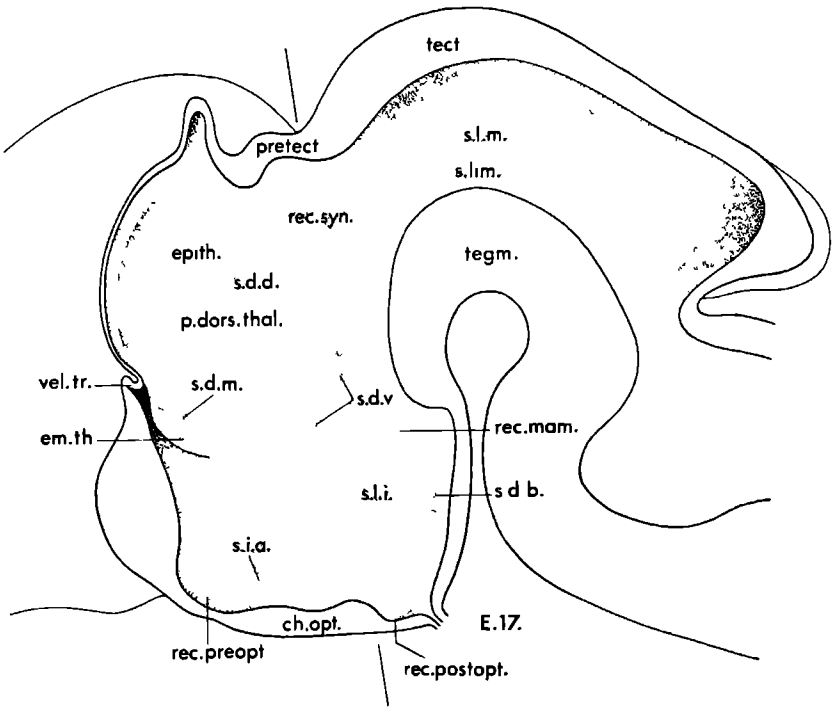


Fig 30 Reconstruction of the ventricular surface in the E_{17} Chinese hamster.

the recessus synencephali is observed. Slightly more dorsally the sulcus diencephalicus dorsalis also ends in the recessus synencephali. This sulcus diencephalicus dorsalis indicates the boundary between the pars dorsalis thalami and the epithalamic region. It is noted that the epithalamus in this stage still occupies a relatively large part of the ventricular wall.

Ventricular aspect at embryonic day 17 (fig. 30)

The ventricular relief is fading. The sulcus intraencephalicus anterior is merely a shallow groove, but the presence of this sulcus and that of the sulcus diencephalicus ventralis, the sulcus lateralis infundibuli and the recessus synencephali can not be denied.

The partes dorsales thalami of both sides meet each other in the median plane. The sulcus diencephalicus medius and the sulcus diencephalicus dorsalis have become rather vestigial. When several specimens of this E_{17}-stage were studied it struck the investigator that the diencephala of some of them have a remarkably oblong and slim outline. This possibly is effected by the increase of

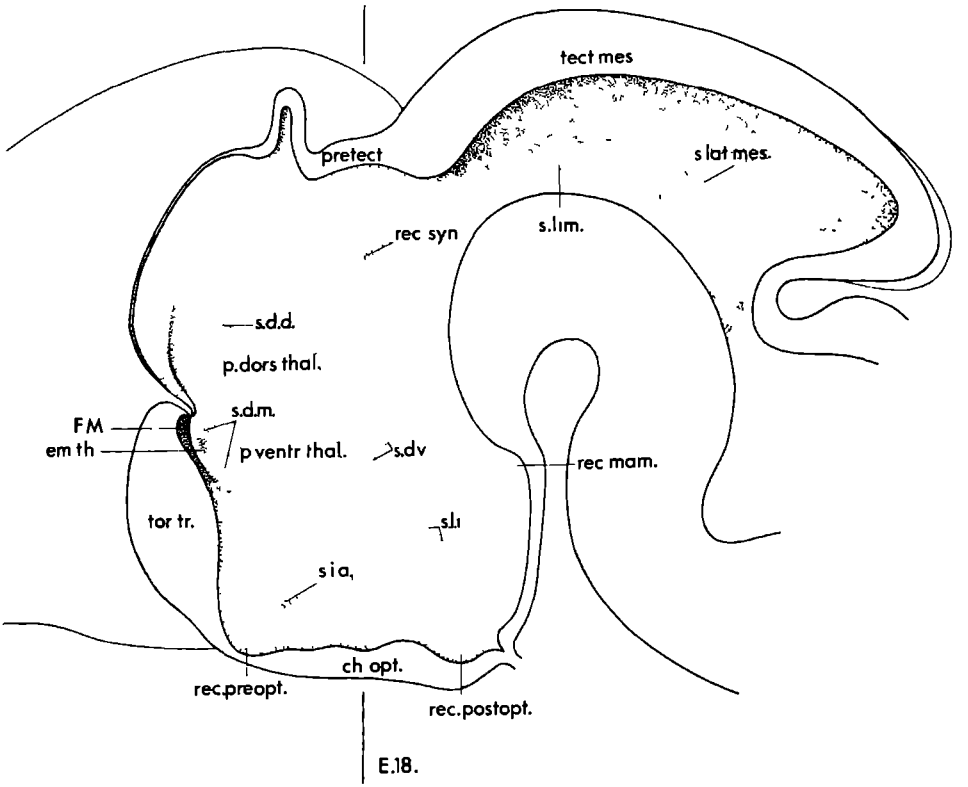


Fig 31 Reconstruction of the ventricular surface in the E_{18} Chinese hamster

the tegmentum mesencephali and by the augmentation in volume of the hemisphere stalk, whereby, in the absence of a pes pedunculi, the diencephalon is squeezed in between.

Ventricular aspect at embryonic day 18 (fig. 31)

The ventricular configuration of this stage approximates the adult aspect closely. It is a "stretching" of the brain that accounts for this fact. This "stretching" consists of an elongation of the hypothalamus. Here the anlage of the corpus mamillare is lowered, presumably by the increase in volume of the tegmentum mesencephali and by the increase in the number of fibres now passing up and down in a position dorsolateral to these structures.

Within the basal part of the diencephalon a sulcus intraencephalicus anterior and a sulcus lateralis infundibuli are clearly recognized. The sulcus diencephalicus ventralis can be followed from the recessus synencephalicus (s. metathalamicus) into the foramen Monroi, where the sulcus terminalis is met.

A sulcus diencephalicus dorsalis and a vestigial sulcus diencephalicus medius also were found within the specimen illustrated here (fig. 31).

As far as the rostral continuation of the mesencephalic sulci is concerned, no evidence could be obtained from the E_{18} series that they reach beyond the synencephalic level.

DISCUSSION

The technique of graphic reconstruction enabled us to obtain an overall picture of the configuration of the ventricular surface at various stages of development. The changes which occur during morphogenesis of the brain could be readily described in this way. For the sake of clarity we will commence the discussion with a summary of our results.

In the youngest stages studied the neural tube is characterized by the neuromeric pattern. A number of neuromeric bulges can be clearly recognized. Ventricularly the neuromeres present themselves as shallow grooves and they are directed, with their greatest dimension, more or less perpendicular to the longitudinal axis of the neural tube (see for instance E_{12}, fig. 18 and E_{13}, fig. 22). A synencephalic and a parencephalic bulge can be indicated clearly, and the latter is already subdivided at the E_{13}-stage, into a posterior and an anterior part. The ventricular relief centering around the optic vesicle and the foramen of Monro is marked at the E_{13}-stage on the one hand by the withdrawal of the optic vesicle from the ventricular wall, and on the other hand by the development of the medial ganglionic hill. The withdrawal of the optic evagination as a part of the neural tube's wall in a lateral direction leads to the formation of a sulcus

which runs into the optic stalk conus from the caudal part of the neural tube. This is the primordial sulcus diencephalicus ventralis. The same process accounts for the origin of a second sulcus running from the optic stalk conus into the hemispheric vesicle. The groove in question is the primary sulcus intraencephalicus anterior, which during later stages communicates with the sulcus terminalis in the caudal part of the lateral ventricle via the foramen of Monro. This primary sulcus intraencephalicus anterior is accentuated at the E_{13}-stage, by the development of the medial ganglionic hill. At this stage and in E_{14} the 'optic' ends of both the sulcus intraencephalicus anterior and of the sulcus diencephalicus ventralis gradually merge. From the E_{14}-stage onward this sulcus being thus from double origin is known as the sulcus intraencephalicus anterior (Von Kupffer, '06). The sulcus diencephalicus ventralis of these early stages has only few points in common with its namesake of later stages. Differences in neuroepithelial development in the lateral wall of the neural tube are very small and the main characteristic of the sulcus in question therefore is that it represents the lateralmost points of the neural tube. The sulcus itself is rather shallow. Later the incongruency that exists between histogenesis of the basal parts of the brain and of the dorsal parts, finds its expression in the accentuation of this early sulcus diencephalicus ventralis.

A sulcus diencephalicus basalis can be observed at the E_{13} and E_{14}-stages, running a longitudinal course next to the basal midline structures, from the region of the recessus inframamillaris towards the postoptic recess. The sulcus lateralis infundibuli that is seen in later stages branching from the sulcus diencephalicus ventralis in a basal direction, is prefigured at the E_{13} and E_{14}-stages as the basal part of the parencephalon anterius cavity.

In E_{15} the appearance of the ventricular surface in the caudal part of the diencephalon is profoundly changed by the disproportionate development of the wall of the parencephalon posterius. This results in the formation of the pars dorsalis thalami. The sulcus diencephalicus ventralis is accentuated between the pars dorsalis thalami and the prerubral tegmentum. The thalamic mass that is protruding into the ventricular lumen replaces the cavity of the parencephalon posterius. The crests that were bordering the parencephalon posterius groove caudally and rostrally at the E_{13} and E_{14}-stages, now are incorporated into the dorsal thalamic mass. At the junction of the pars dorsalis thalami and the epithalamus a sulcus can be observed. This sulcus is called sulcus diencephalicus dorsalis, and its rostral moiety probably represents a remnant of the parencephalon posterius cavity. In E_{16} it can be followed caudally into the synencephalic recess. Within the dorsal part of the parencephalon anterius a shallow remnant of the original cavity remains to be seen in E_{15} and it is almost certain that this sulcus has been called sulcus diencephalicus medius by Kuhlenbeck in the pig embryo ('30). Thus, at the E_{16}-stage all eventual morphological features of the ventricular surface of the diencephalon have developed. In subsequent

days a relative obliteration of the ventricular lumen takes place. This is effected by the increasing mass of the lateral walls of the diencephalon. The area that is enclosed by the sulcus lateralis infundibuli, sulcus diencephalicus ventralis and sulcus intraencephalicus anterior increases its surface and the depth of the sulci just mentioned decreases; no essential changes, however, take place in the configuration of the ventricular relief.

The ever increasing development of the pars dorsalis thalami at later stages causes the relative displacement of the parencephalon-anterius-derived pars ventralis thalami into a lateral direction. By this displacement the sulcus diencephalicus medius (originally representing a dorsal part of the cavity of the parencephalon anterius) and the sulcus diencephalicus ventralis (originally crossing the parencephalon anterius in a more or less right angle) become undiscernable. Henceforth they are represented by the sulcus hypothalamicus s. Monroi that forms the ventricular border between a dorsal, thalamic and a basal, hypothalamic part of the diencephalon.

Let us now turn to the answering of the questions posed at the end of the introductory paragraph of this chapter. First the question of the relationships between neuromeres and ventricular sulci will be discussed. Next, the morphological position of the so-called 'horizontal' diencephalic grooves which separate the four stories will be debated. In the third place the sulcus limitans is the object of our discussion.

Relationships between neuromeres and ventricular sulci

The question of a possible relationship between neuromeres and the sulci observed on the ventricular surface of older stages can now be answered. Early neuromerism finds its expression in the origin of a particular shape of the neural tube, as we have seen in chapter IV. This shape consists of an internal and an external relief that in the early stages is almost equal and similar. As development proceeds, the increase in volume of the ventricular wall causes the disappearance of this similarity of outer and inner surfaces, and the ventricular surface obtains its own configuration. It is evident that both the neuromere-caused relief and the sulcal pattern of later stages are in part caused by the histogenetic processes occurring in the wall of the neural tube. In fact it was already argued in 1926 by Bartelmez and Evans, and in 1932 by Bergquist, that at the neuromere stages many of the bulges and sulci occur at the sites of major proliferative activity. These sulci were even called "proliferation-grooves" (Proliferationsfurchen) by Bergquist. In later years Källén ('51) and Bergquist and Källén ('54) studied the cause of neuromeric bulges and ventricular sulci in some detail. They proved the coincidence of proliferation maxima with the site of largest measurement of the neuromeric bulges. Our results are in accordance with these observations.

In our opinion the authors mentioned above failed, however, to give a plausible explanation of the fact that at later stages ventricular sulci frequently form boundary lines for the structures that develop within the ventricular wall. It is this attribute of ventricular sulci that has led Herrick ('10, '33) to accept them as a criterion to infer homologies within the vertebrate diencephalon. As we have seen in chapter IV, the waxing and waning of proliferative activity as expressed by the occurrence of an increasing number of neuromeric bulges in the proneuromeric and neuromeric phases of development, comes to an end with the start of the postneuromeric period; by then, differentiation has started in the basal parts of the interneuromeric crests, while the proliferative activity remains maximal at the centre of the neuromere. Hence, as a consequence of the difference between the rate of proliferation at the border and at the centre of the neuromere, the gradual thickening of the wall of the neural tube in some cases causes the fading of the neuromeric groove and even the bulging of the central part of the neuromere into the ventricular lumen. By that time the original ventricular interneuromeric eminences may have been transformed into ventricular sulci. This mechanism, however, accounts only for one out of several possible ways of formation of ventricular sulci. It is illustrated by some

Fig 32 Schematic representation of the neuromere-derived ventricular sulci. The Roman numerals are indicative of the corresponding neuromeres (cf. fig. 16b).

of the sulci that border the pars dorsalis thalami (the posterobasal part of the sulcus diencephalicus dorsalis in E_{17}, fig. 30; the sulcus diencephalicus medius in E_{15}, fig. 27).

A number of diencephalic sulci, however, can be interpreted as forming the

relics of the original neuromeric grooves; in figure 32 a summary is given of this kind of sulci. Our observations provide evidence which makes it warranted to conceive part of the sulcus intraencephalicus anterior as a relic of the optic neuromere. The basal part of the parencephalon anterius is represented at later stages by the sulcus lateralis infundibuli; the parencephalon posterius remains as the anterior part of the sulcus diencephalicus dorsalis; and finally the synencephalic cavity remains as the recessus of the same name.

Another factor in the formation of sulci is the transformation of the ventricular relief caused by the mechanical forces exerted by a different rate in development prevailing in the basal and dorsal regions. The dorsal part of the sulcus intraencephalicus anterior is an example of this kind of groove and the sulcus diencephalicus ventralis is another one.

Thus, in our opinion, several processes contribute to the formation of the ventricular relief. One single cause cannot be incriminated and the relations between the ventricular sulci and the structures in the neural tube's wall in some instances change according to the stage of development in which they are studied.

The morphological value of the ventricular sulci of the diencephalon

In literature, the opinion is expressed that the main diencephalic grooves (*i.e.,* sulcus diencephalicus dorsalis, sulcus diencephalicus medius and sulcus diencephalicus ventralis) have a position that is morphologically speaking longitudinal (His, 1890; Herrick, '10, '17; Kuhlenbeck, '27). Our observations make it plain that a true longitudinal position can only be attributed to the sulcus diencephalicus ventralis. The sulcus diencephalicus dorsalis and the sulcus diencephalicus medius are, in part at least, derived from morphologically transverse, neuromeric grooves. The same applies for the sulcus lateralis infundibuli. This morphologically transverse position of many of the diencephalic sulci was advocated earlier by Haller ('29) and Bergquist ('32) and we are able to confirm their conclusions fully.

The sulcus limitans question

As was mentioned in the introductory paragraph of this chapter, a number of authors have expressed their opinion on the rostral continuation of the sulcus limitans beyond the mesencephalon into the prosencephalon. They all took their starting point in the assumption that the bipartite lateral plate of the mesencephalon, which is subdivided by the sulcus limitans into a motor basal plate and a sensory alar plate, continues beyond the mesencephalon. This extension would equally subdivide the prosencephalon into basal and alar plate derivatives. To check the validity of this assumption is beyond the scope of this

study. We will try to find an answer to the question whether the mesencephalic sulcus limitans can be followed beyond the di-mesencephalic border in a rostral direction.

The identification of the sulcus limitans within the mesencephalon is not a matter of self-evidence. His (1893[a]) introduced this term for the adult human brain but stated that the position of this sulcus in the adult is not exactly the same as that of the embryonic sulcus limitans In Palmgren's ('21) study on the development of the mesencephalon in which he studied the mouse, the sulcus lateralis tecti (= our sulcus lateralis mesencephali) was the only remaining groove in the adult mouse. The groove which was observed in a number of embryos basal to this sulcus lateralis mesencephali was called by Palmgren sulcus lateralis, as he was reluctant to apply a term as vaguely defined as the sulcus limitans, to this groove. From the study by Bengmark, Hugossen and Kallén ('53) it is clear, however, that the sulcus lateralis of Palmgren can safely be concluded to form the dorsal border of the functionally motor part of the mesencephalon.

The sulcus limitans of His's interpretation thus being identified in the Chinese hamster brain, a second question can be answered now: is it possible to follow this sulcus limitans from the mesencephalon into the diencephalon? According to many authors this sulcus corresponds to the sulcus diencephalicus ventralis (His, 1893[a]; Johnston, '09; Streeter, '11; Spatz, '27; Bergquist, '32; Grunthal, '52). Others forwarded the opinion that the sulcus ends with rostrodorsal convexity in the mamillary region (Schulte and Tilney, '15; Kingsbury, '20, '22; and Kuhlenbeck, '27).

A continuity of the mesencephalic sulcus limitans with a more rostrally located diencephalic sulcus could not be demonstrated in this study. At the earliest stages (E_{12}, fig. 17; E_{13}, fig. 21; E_{14}, fig. 24) the lateralmost points of the ventricular surface together form a kind of longitudinal groove. The relief in these stages, however, is so smooth that this groove hardly deserves a denomination as sulcus and certainly no relation with a basodorsal difference in development can be observed (as the sulcus limitans is supposed to mark).

The presence of the recessus synencephalicus (in whose cavity both mesencephalic sulci widen and disappear) prevents one from coming to a conclusive decision about the existence of a continuity between the mesencephalic sulci and the sulcus diencephalicus ventralis which takes origin from the rostral side of the synencephalic recess.

The third question about the rostral continuation of the sulcus diencephalicus ventralis can be answered in a more satisfactory way. In early stages, the primordial sulcus diencephalicus ventralis originates as a longitudinal sulcus that continues into the optic stalk conus and ends in the optic vesicle (E_{13}, fig. 21). Later on, the primary sulcus intraencephalicus anterior which is present in the stages just mentioned, concurs with the rostralmost part of the sulcus

diencephalicus ventralis as a secondary consequence of the development of the surrounding neuroepithelial structures. Thus, at this stage (E_{14}) the sulcus diencephalicus ventralis can be followed both into the recessus preopticus through the sulcus intraencephalicus anterior and into the lateral ventricle by way of the foramen of Monro. Neither possibility is favoured over the other one as at this stage the site of confluence of the sulci is rather flat and has a triangular shape. By the development of the adjacent ventricular wall in later stages, the sulcus intraencephalicus anterior becomes less and less clear. The sulcus diencephalicus ventralis obtains a rather impressive accentuation by the disproportionate development of the thalamic part of the diencephalon, by which also the eminentia thalami is formed. The rostral continuation of the sulcus diencephalicus ventralis in the final stages of morphogenesis therefore is formed by the sulcus running into the foramen Monroi. This sulcus is continuous with the sulcus terminalis of the lateral ventricle. Thus it becomes clear that in the mammal under study, the rostral continuation of the sulcus diencephalicus ventralis depends entirely upon the stage in which the observations are made. Thereby an explanation is offered for the disagreement existing among the authors as mentioned above. This conclusion is in accord with the study published by Coggeshall on the rat's diencephalon (Coggeshall, '64).

CONCLUSION

In this chapter we have demonstrated a circumscript relation between the postneuromeric ventricular relief and the eventual ventricular sulci which characterize the configuration in later stages of ontogenesis and adulthood. A number of sulci derived from neuromeric bulges has a morphologically transverse course; the sulcus diencephalicus ventralis on the contrary, has a longitudinal course and is effected largely by the mechanical consequences of a difference in development between basal and dorsal parts of the neural tube.

Also we observed that in the case of the sulcus diencephalicus dorsalis and sulcus diencephalicus medius the localisation of the sulcus in relation to the structures in the ventricular wall changes according to the stage of development under study.

The heterogeneity of factors leading to the development of the diencephalic sulci hardly leaves any doubt as to the unreliability of grooves in the ventricular relief as a criterion of subdivision within the diencephalon during ontogenesis. This raises the question of the validity of either the neuromere-based attempts to set up a "Bauplan" of the diencephalon (Haller, '29; Bergquist, '32; Bergquist and Källén, '54) or of the validity of the His-Herrick-Kuhlenbeck scheme, which is based essentially on the occurrence, in the adult form, of four diencephalic longitudinal cell columns or 'floors' (epithalamus, thalamus dorsalis,

thalamus ventralis, hypothalamus), demarcated by ventricular sulci (sulcus diencephalicus dorsalis, sulcus diencephalicus medius, sulcus diencephalicus ventralis).

In the following chapters the relationship between the histogenetic phenomena and the resulting neuronal organization on one hand and the data obtained from the study of morphogenesis as described in chapters IV and V on the other, will be matched in order to find a solution of this question.

CHAPTER VI

HISTOGENESIS WITHIN THE DIENCEPHALON: THE MATRIX

INTRODUCTION

Present day concepts of cell behaviour in the developing nervous system show important modifications of the classical ideas formulated by His in 1889. These concepts have a profound bearing on our understanding of the histogenetic processes within the brain and we will discuss this subject to some extent.

The cells that constitute the neural plate and the wall of the neural tube initially exhibit an epithelial arrangement, the epithelium presenting the pseudo-stratified columnar variety. In this relatively simple epithelium His distinguished between an inner zone (Säulenschicht), an intermediate zone, containing the epithelial nuclei, and an outer zone (Randschleier). The first and the third both consisted of cytoplasmatic processes of the epithelial cells. The inner zone in addition contains the so-called germinal cells or "Keimzellen" (see below).

The basic concept in His's view on the histogenetic process was the assumption of the existence of two essentially different populations of cells in this neuroepithelium. The epithelial cells, according to His, are to be transformed into spongioblasts, presumptive glial cells that, possessing an internal and an external process, are arranged radially to the main axis of the neural tube. These spongioblasts thus provide a glial framework (Markgerüst) and are forming, by their internal processes, a membrana limitans interna at the ventricular side and they are fixed, by their external processes, to the innermost part of the Randschleier or marginal layer. Within this marginal layer the processes of several spongioblasts are continuous, thus forming a syncytial structure. The second group of cells consists of the germinal cells (Keimzellen) that are located within the juxtaventricular "Säulenschicht", and which are surrounded by the internal spongioblastic processes. In His's opinion the germinal cells are mitotically active and their offspring consists of neuroblasts that are bound to migrate as an intermediate form (within the preformed spongioblastic framework) into a peripheral direction to settle just internal to the marginal layer or Randschleier. By this process of neuroblast formation, the intermediate zone becomes divided into an external mantle layer, containing neuroblasts, and an internal "Innenplatte", consisting of the crowded nuclei of spongioblasts and of young migrating neuroblasts. Later on the Innenplatte gradually diminishes in thickness, by the progressive differentiation of the migrating neuroblasts, to the profit of the dimension of the mantle layer.

This 'classical' concept of His was questioned by Schaper (1897) already and

also by F. C. Sauer ('35a, '35b, '36, '37). The latter concluded "that the radially arranged columnar cells, designated by His (1889) as spongioblasts, and the rounded cells in stages of mitosis, which His named germinal cells, are not two types of cells, but are the interkinetic and mitotic stages of the same cell" (Sauer, '35a, p. 393). Thus, according to Sauer, within the neural plate and within the wall of the early neural tube, one single homogeneous population of cells exists, but they are in different stages of the mitotic cycle. The typical ventricular position of the mitotic figures was accounted for by the firm attachment of all epithelial cells to a 'terminal web', located at the ventricular surface. The rounding up of the cell in the final stages of the mitotic cycle causes it to be forced towards the ventricular surface, and after completion of the division the daughter nuclei are allowed to migrate peripherally to initiate a new cycle. F. C. Sauer's results were confirmed by a vast number of studies using different techniques, among which autoradiography and studies determining the D.N.A. content of nuclei deserve mentioning (Duncan, '57; Bellairs, '59; M. E. Sauer and Chittenden, '59; M. E. Sauer and Walker, '59; Sidman e.a., '59; Watterson e.a., '56; Fujita, '62; Källén and Valmin, '63; Lyser, '64).

The purely epithelial structure of the homogeneous population of neuroepithelial cells, ceases to be so by the entrance of blood vessels and by the appearance of neuroblasts in the wall of the neural tube. By then a peripheral marginal layer can be distinguished, consisting of cytoplasmatic processes of the epithelium (Bergquist, quoted by Källén, '65) and central to it a mantle layer is observed, containing neuroblasts. The ventricularly located neuroepithelial cell population, that continues its proliferative activity during this incipient differentiation, is called the matrix layer (Rose, '42; Kahle, '51). The matrix cells, in their interkinetic state, continue to extend their processes towards both the ventricular surface and the marginal layer of the neural tube.

Apparently this matrix layer corresponds to both His's Innenplatte and germinal cells, and to the neuroepithelial layer or primitive ependymal layer of other authors (Cooper, '50; Dekaban, '54; Langman, e.a. '66). Recently, the term 'ventricular zone' was proposed by a self-appointed Boulder Committee ('69). In this topographic name, however, the dynamic connotation of the matrix (= maternal) layer, as giving birth to generations of neurons and, probably, glial elements, is entirely lost. The Boulder Committee objected that the term matrix did not account for the existence of a subependymal layer as is encountered in the subpallial and pallial regions. As the universal occurrence of a subependymal layer remains to be demonstrated, much credit to this objection can not be given.

The transformation of the purely neuroepithelial neural tube into the vascularized primordial nervous tissue is but the first step in a whole range of differentiation-events to which we will recurr in later chapters. Kahle ('51, '56, '58) has described the natural history of the matrix layer within the human diencephalon

The matrix layer, after having given origin to numerous neuronal and probably glial elements, becomes exhausted and remains as an ependymal layer. Within the mantle layer, that increases *pari passu* with the exhaustion of the matrix layer and that is composed largely of the offspring of the matrix cells, the grisea of the central nervous system originate by migration and regrouping of neuroblasts. The outgrowth (in specific patterns) of axonal and dendritic processes from the maturing neuroblasts transforms the neural primordium into the organ of integrative activity as it is met with in the adult form.

Summarizing this review of literature, it can be concluded that the neurogenetic process is rather articulate. The early neuroepithelium first develops into a homogeneous cell population, all cells actively taking part in a proliferation process; they are distributed randomly along the mitotic cycle. Within this pseudo-stratified epithelium the cells are anchored firmly to the ventricular terminal web, and during their interkinetic phase, perform an elevator movement to and from the ventricular surface. Mitotic spindles are only observed in the immediate vicinity of the ventricular surface. This proliferation continues after the appearance of the first neuroblasts that constitute a mantle layer external to the matrix layer. The latter phenomenon is caused by the radial migration of the differentiated cells. The mantle layer gradually thickens and finally acquires a preponderance as compared to the matrix layer that becomes exhausted. Within the mantle layer the axon of the neuroblast and its dendritic tree develop. The myelination of the axon constitutes the final stage of neuronal differentiation. In these later phases of neurogenesis the relationships are established that exist in the adult brain between its constituent parts.

Thus, this survey shows us that the microscopic picture of the wall of the neural tube, as observed by the anatomist during various stages of development, represents no more than an instantaneous snapshot of a highly complex process. The causal factors underlying this development can hardly be touched upon in the present context.

The processes described above, although uniformly programmed in their mutual sequence, do not take place at the same time in different parts of the central nervous system. They are temporo-spatially patterned. This heterochrony in rate of development of the neuroepithelium causes the appearance of a "naturliche Selbstzergliederung" (natural self-subdivision) (Flechsig, 1896) that gradually reveals itself within the wall of the developing nervous system. Heterochrony within the developing neural tube can be observed as expressing itself in several phenomena. Firstly, the proliferative activity in the early neural tube shows maxima that can be demonstrated to coincide with the bulgings of neuromeres (Kallén, '52, '53), as was discussed in the chapter on neuromerism. It is an attractive hypothesis to relate the phenomenon of neuromerism to the existence of proliferation maxima and these maxima in their turn to heterochrony within the wall of the neural tube.

Another phenomenon that is characterized by heterochrony is the differentiation of neuroepithelial cells into neuroblasts. The appearance of neuroblasts peripheral to the matrix layer is first demonstrable within the rhombencephalon, as can be judged from the development of neurofibrils (Windle and Baxter, '35, rat; Windle and Austin, '35; Lyser, '66, chick). From there on it is found spreading into both the rostral and the caudal direction. The differentiation phenomenon is accompanied by the disappearance of the neuromeres and shows a patterned aspect; it results in the development of 'Grundgebiete' (Bergquist, '32) or 'migration areas' (Bergquist and Källén, '54) that are subdivisions of the so-called transversal bands or postneuromeres.

A third phenomenon characterized by heterochrony is the ultimate exhaustion of the matrix layer, which shows a pattern quite different from that seen in the origin of the migration areas. This matrix exhaustion was studied by Kahle ('51, '56) in the human diencephalon. In general, if an inquiry is made into the sequence of matrix exhaustion in different regions of the neural tube, both a basodorsal and a caudorostral gradient are observed. The basodorsal gradient was known already by the work of His ('04), Kölliker (1896) and Streeter ('11), who observed a striking difference in degree of development between the alar and basal plate areas. The caudorostral gradient was highlighted for the first time by Spatz ('27), who was struck by the lagging behind of the telencephalon, when compared to the diencephalic development.

Kahle's investigation on heterochrony phenomena in the human embryonic nervous system seemed to warrant a generalization of this observation for the whole brain (Kahle, '51). In a second publication (Kahle, '56) this author focussed his attention on the diencephalon. He concluded to the existence of a caudorostral and a basodorsal gradient in the diencephalon too but he noted that within this basic pattern a number of exceptions exists. The first signs of differentiation are observed within the subthalamus, *i.e.*, the dorsal part of the hypothalamus or the 'entopeduncular group' (Kuhlenbeck, '48, '54). The pars dorsalis thalami shows the process of differentiation exceedingly late, and both the basal part of the hypothalamus and the epithalamus are characterized by a rather prolonged presence of matrix phases. In this chapter the conclusions of Kahle will be tested in the Chinese hamster as far as the matrix is concerned. The differentiation of the mantle layer will be dealt with in the two subsequent chapters.

TECHNIQUE

In order to obtain a readily communicable picture of the matrix condition within the diencephalon at different stages of development as a basis for the comparative study of these stages, it was decided to map out the functional state of the matrix on the ventricular surface. This method involves the following

steps: (1) The developmental history of the neuroepithelium was subdivided into nine phases (fig. 33a). This subdivision is marked by a certain arbitrariness as the process of matrix development actually shows a rather continuous progress. (2) Every tenth section of a series was drawn on a sheet of paper with the help of a projection microscope and under microscopical controll the phases of development of the neuroepithelium were indicated along the ventricular surface (see fig. 33b). (3) With the help of the drawings just mentioned, graphic reconstructions were prepared by projection on the median plane. Using a code, then, the differences of the functional state of the neuroepithelium were visualized in the maps thus obtained. Care was taken to avoid misinterpretations in those places where the matrix was not cut perpendicularly to the ventricular surface.

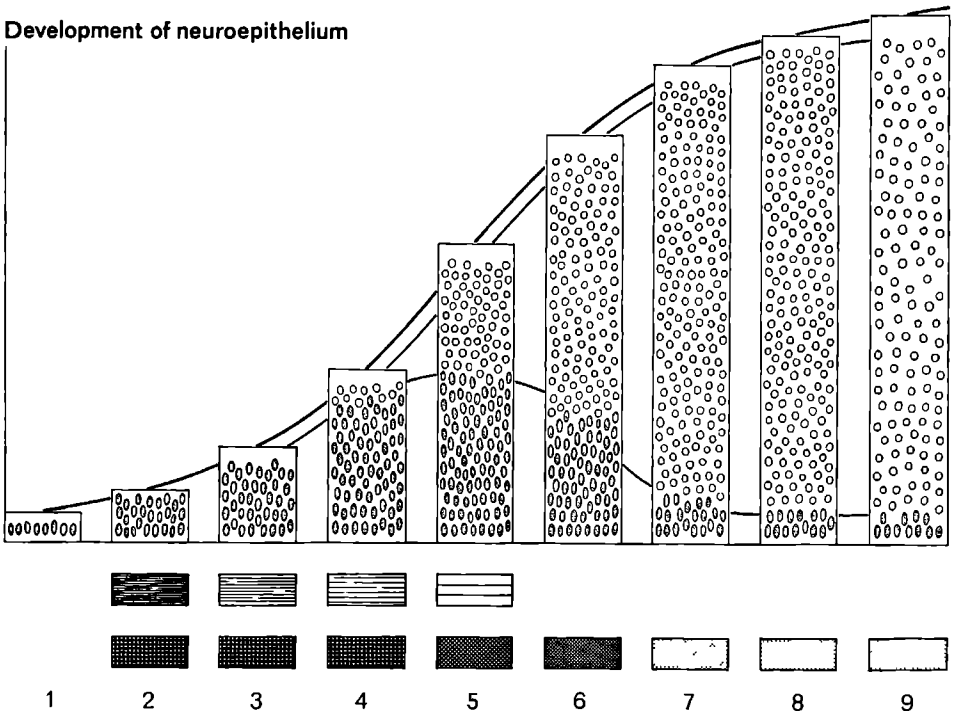


Fig. 33a. The development of the neuro-epithelium. The natural history of the development of the matrix is subdivided into nine phases (cf text). The blocks under the base-line of the figure represent the code employed for the various developmental phases in the reconstructions shown in figures 33b-47.

The subdivision of the developmental history of the neuroepithelium in nine phases was performed as follows (see also fig. 33a);

1. In the neural plate and in the very early neural tube a monolayered neuroepithelium was observed.

2. From this primitive state a pseudo-stratified epithelium originates.
3. Later on a small marginal layer develops peripheral to the pseudo-stratified neuroepithelium.
4. This pseudo-stratified neuroepithelium, possessing a marginal layer, then becomes vascularized.
5. In between the matrix and the marginal layer a mantle layer appears.
6. This mantle layer becomes gradually thicker and when it is as wide as the matrix we consider the process of maturation as reaching phase 6.
7. The matrix approaches exhaustion, but still a number of mitoses remains to be seen.
8. Matrix-exhaustion follows; along the ventricular wall no mitoses are observed. The area bordering on the ventricular surface, however, retains its rather basophilic characteristics.
9. The ventricle bordering area looses its compact aspect and, presumably by the increase in mass of the neuropil, is less basophilic in the H.E.-preparation. By now, the ventricle is lined with an ependymal layer.

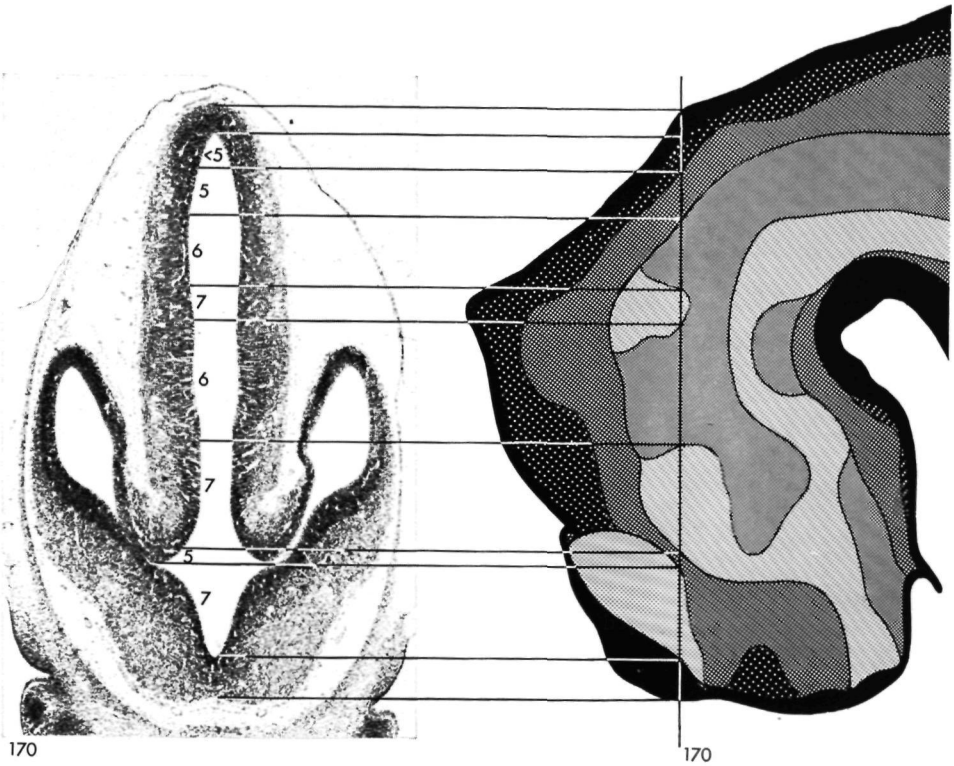


Fig. 33b. Schematic representation of the technique employed in the preparation of matrix-phase maps.

As all phases just described represent a definite relationship between a functional state of the matrix layer and other structures within the developing neural tissue, we adopted Kahle's term "Matrix Phases" to designate these stages.

At least four specimens from each day of development were phase-typed and plotted on a graphic reconstruction of the midsagittal section of the diencephalon. A selection of the maps, that were obtained in this way, illustrates this chapter.

OBSERVATIONS

The description of the matrix phase maps starts at embryonic day 12. Prior to this stage of development the neural tube is dominated by the early neuromeric pattern, showing no impressive differences in histologic appearance.

Matrix phases at embryonic day 12 (figs. 34 and 35)

At this stage of development the wall of the neural tube shows a rather simple condition. It consists mainly of a pseudo-stratified epithelium and possesses a narrow marginal layer. Within the rhombencephalon and the tegmental areas vascularization of the neuroepithelium has occurred. This progressive basal area extends into the diencephalon dorsal and rostral to the tuberculum posterius, the more advanced phases being restricted to the basal half of the brain. This area of early maturation does not reach the optic stalk, which is surrounded by a less advanced type of epithelium. In the eminentia interneuromerica between the telencephalon and the optic neuromere an island of further differentiation is observed. A most conservative behaviour is displayed by the neuroepithelium of the midline regions adjacent to the velum transversum, the infundibulum and the optic chiasm. Both reconstructions show a fairly consistent picture.

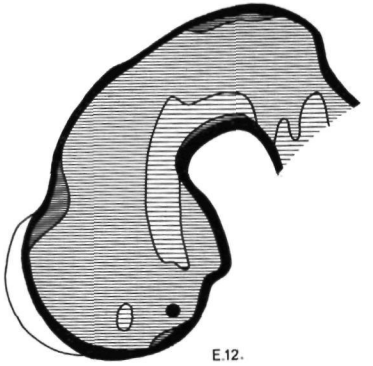


Fig. 34 Map showing the development of the matrix at E_{12} (cf. fig. 18).

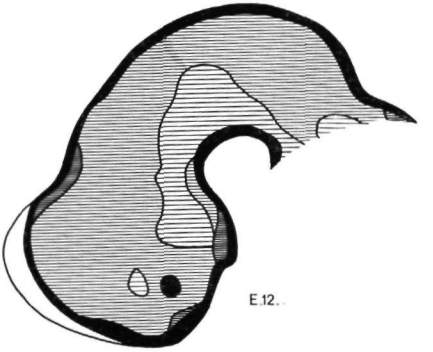


Fig. 35 Map showing the development of the matrix at E_{12} (cf. fig. 17).

Matrix phases at embryonic day 13 (figs. 36, 37 and 38)

When compared to E_{12} progress has been made at this stage mainly in the basal areas. The tegmental region shares its leadership with the area that has been called by Gilbert ('35) "hypothalamic cell cord". This runs from the

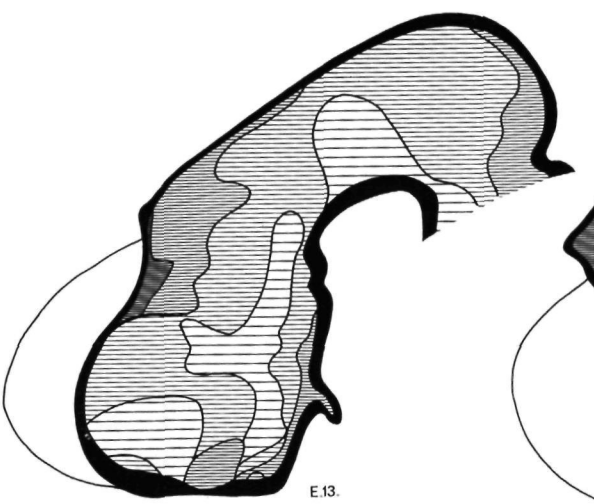


Fig. 36 Map showing the development of the matrix at E_{13} (cf. fig. 20).

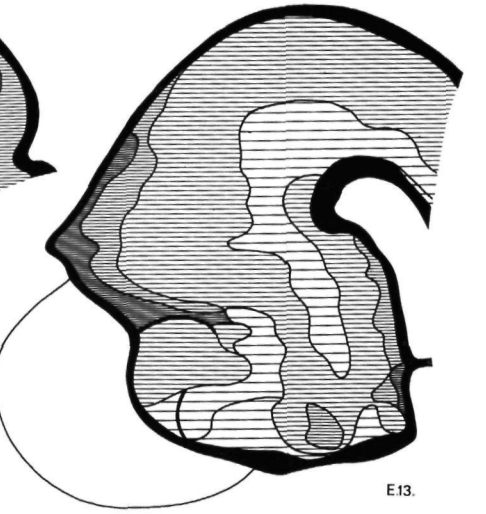


Fig. 37 Map showing the development of the matrix at E_{13} (cf. fig. 21).

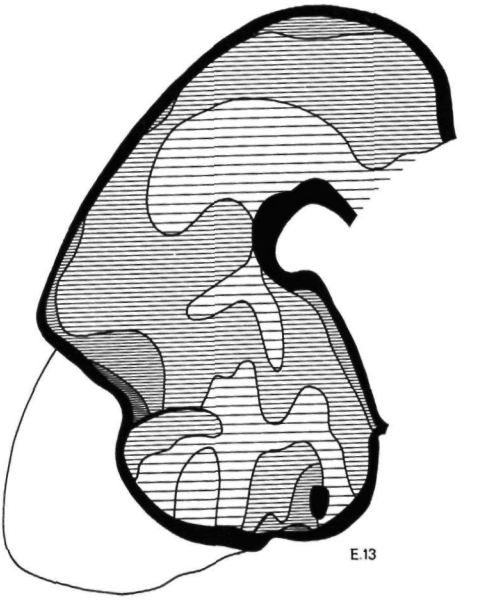


Fig. 38 Map showing the development of the matrix at E_{13} (cf. fig. 22).

tuberculum posterius region towards the future chiasmatic plate. From these basal, longitudinal areas, several tongue-like extensions reach towards the convexity of the neural tube. They concur with the basal parts of the earlier eminentiae interneuromericae between synencephalon and parencephalon posterius, between parencephalon posterius and parencephalon anterius and between parencephalon anterius, optic and telencephalic neuromeres. Not all basal parts of the regions of the brain now under consideration are accelerated in development. The area located basal to the hypothalamic cell cord and also the one immediately rostral to the optic stalk (regio preoptica) are retarded considerably. The area centering around the velum transversum also shows a lack of progress. Parenthetically it may be briefly stated here that also the colliculus posterior displays a retardation when compared to more rostral tectal and pretectal areas.

Matrix phases at embryonic day 14 (figs. 39, 40 and 41)

Between E_{13} and E_{14} considerable changes have taken place. On the whole the basal parts are still accelerated and the early phases, commonly encountered over large areas at the E_{13}-stage, by now are limited to the basal hypothalamic area, the preoptic region and the convex part of the diencephalon and mesencephalon. A curious phenomenon at this stage is the relative retardation of the tuberculum posterius region, that in this respect resembles somewhat the hypothalamic area.

Fig. 39 Map showing the development of the matrix at E_{14} (cf. fig. 23).

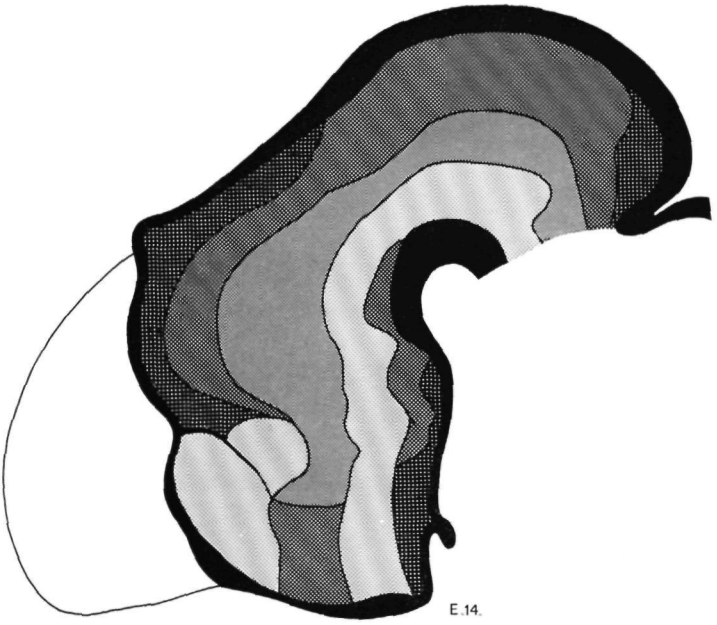


Fig. 40 Map showing the development of the matrix at E_{14} (cf. fig. 24).

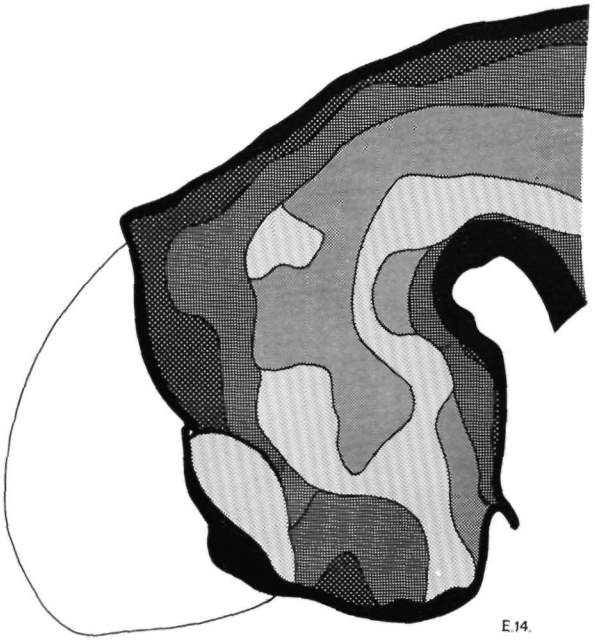


Fig. 41 Map showing the development of the matrix at E_{14} (cf. fig. 25).

The most progressive areas form an extension of those already observed in the previous stages: *i.e.*, the tegmental and hypothalamic cell cord regions, a region at the base of the thalamic eminence and furthermore the medial ganglionic hill and the rostral part of the preoptic region. A striking feature is the disappearance at this stage of the tongue-like formations that could be seen in earlier specimens. Especially if one takes together the two codes representing the most progressive phases within this stage, it looks as if the intervening space has succeeded in keeping up with its neighbourhood; thus a broad progressive zone has developed, dichotomizing in the more rostral parts of the diencephalon to reach the chiasm and the medial striatal eminence basally and rostrally.

Matrix phases at embryonic day 15 (figs. 42 and 43)

At this stage the first exhausted matrix area shows up in the tegmental mesencephalic area. The most retarded regions, on the other hand, all the same occupy the areas mentioned in the E_{14}-stage. We also reconstructed an E_{15} specimen that, when compared to its equals in age, showed an advanced development (fig. 43). In this specimen the retardation of the pars dorsalis thalami is clearly illustrated. This region is surrounded by a zone of progressive development. In its caudal and dorsal parts this zone corresponds with the future fasciculus retroflexus and part of the epithalamus respectively. Rostrally it is represented in later stages by the pars ventralis thalami and the subthalamic structures.

Matrix phases at embryonic day 16 (figs. 14 and 45)

A conspicuous feature in the E_{16} specimens studied is the relative immaturity of the dorsal thalamic matrix; it lies isolated in the midst of accelerated matrices. These accelerated matrices occupy the diencephalic part of the tegmentum, the future subthalamic cell masses, the progressive pars ventralis thalami and the interneuromeric crest in which the fasciculus retroflexus is developing. Less advanced areas are: the hypothalamus basal to the hypothalamic cell cord, the preoptic region, the eminentia thalami, the habenula (except its most caudal part), the epiphysis and the colliculus inferior. Thus, a steady progression is taking place, exempting however those midline structures, which are not crossed by commissural or decussating fibre bundles.

Matrix phases at embryonic day 17 (fig. 46)

The area in which matrix exhaustion has occurred is increased considerably and covers a large part of the pars dorsalis thalami also. The regio preoptica and the basal hypothalamic area is maturing by now and so are the epithalamus and the colliculus inferior. These regions, however, are still retarded when compared to the other diencephalic and mesencephalic areas.

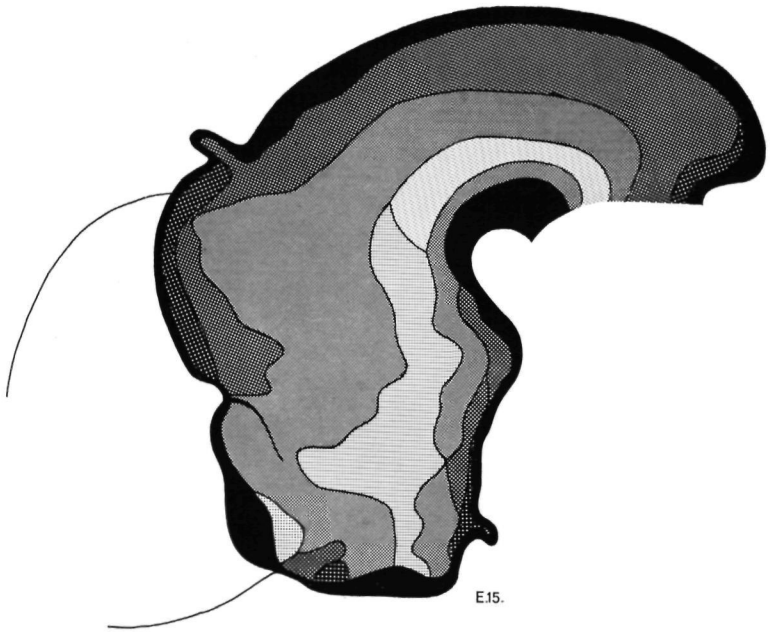


Fig. 42 Map showing the development of the matrix at E_{15} (cf. fig. 26).

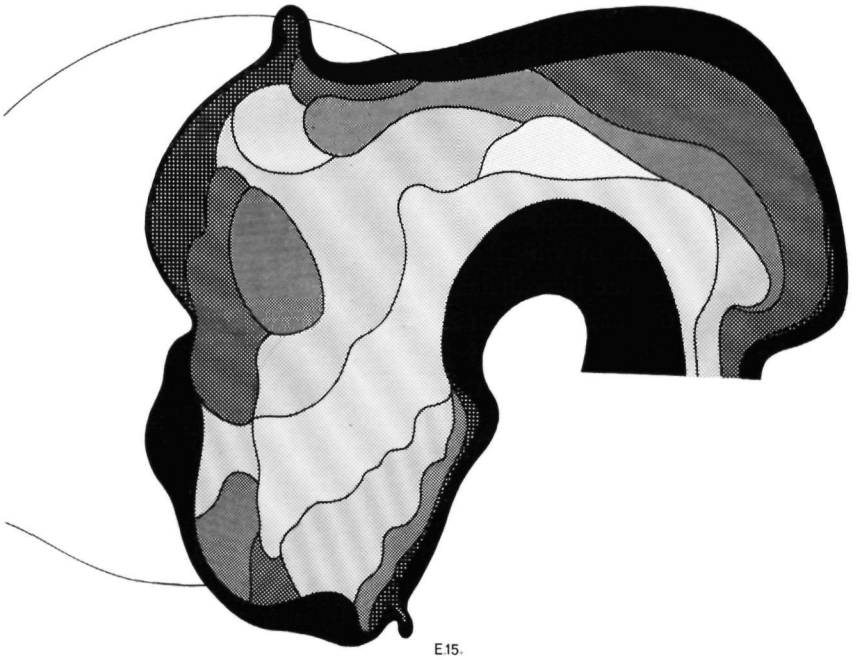


Fig. 43 Map showing the development of the matrix at E_{15} (cf. fig. 27).

Fig. 44 Map showing the development of the matrix at E_{16} (cf. fig. 29).

Fig. 45 Map showing the development of the matrix at E_{16} (cf. fig. 28).

E17

Fig. 16 Map showing the development of the matrix at E_{17} (cf fig 30)

Matrix phases at embryonic day 18 (fig. 17)

At this stage there is an almost general exhaustion of the matrix. The only places where mitoses are still observed are: the epithalamus, the regio preoptica, the basal part of the hypothalamus and the colliculus inferior.

DISCUSSION AND SUMMARY

Kahle ('51, '56, '58) is the only author who has made an extensive study of the matrix development within the diencephalon of a mammalian embryo. He studied human material and was mainly interested in those stages of development in which a mantle layer was observed. His point of departure was the classical concept of neural histogenesis as expressed by His (1889). In his studies he confined himself to relatively late stages that is to say, stages in which a mantle layer is observed. In the histogenesis of the wall of the neural tube he discerned six phases, three of them representing phases of increasing mantle layer production (*i.e.*, beginning, advanced and highest rate of migration of matrix cells in a peripheral direction) and three phases representing decreasing proliferative activity in the matrix (*i.e.*, beginning, advanced and completed exhaustion).

Fig. 17 Map showing the development of the matrix at E_{18} (cf. fig. 31).

In the present study Kahle's results, obtained from human material, will be compared to those obtained from embryos of the Chinese hamster. For the sake of clarity, however, three remarks should be made on the comparability of the work of Kahle with this study: (1) We decided to start our study at a considerably earlier phase of development, before the appearance of a mantle layer. In fact our study encompasses the development of the diencephalon of the Chinese hamster from the early closure of the anterior neuropore onwards. (2) Many important data on the histogenesis of the nervous system were not yet known at the time Kahle carried out his studies. Thus we were enabled to reformulate the "matrix phases" on the basis of data obtained with the help of modern techniques (M. E. Sauer and Chittenden, '59; M. E. Sauer and Walker, '59; Fujita, '62). (3) Another important point is the fact that Kahle did not test the validity of the concept of His-Herrick-Kuhlenbeck, according to which the diencephalon is subdivided into four stories (epithalamus, pars dorsalis thalami, pars ventralis thalami, hypothalamus) by three longitudinal sulci. Rather he took this subdivision as the starting point for his study. In our study, on the contrary, the rather fundamental question is posed whether there is a relation-

ship between the ventricular relief and the phenomena of neuromerism on the one hand and the rate of development of the neuroepithelium on the other.

We will now turn to a discussion of the ontogenesis of the Chinese hamster diencephalon from the point of view that is allowed by the examination of histogenesis in the material at our disposal. From the data presented above, it may be concluded that as far as matrix development is concerned in the diencephalon both a caudorostral and a basodorsal gradient can be discerned (see fig. 48).

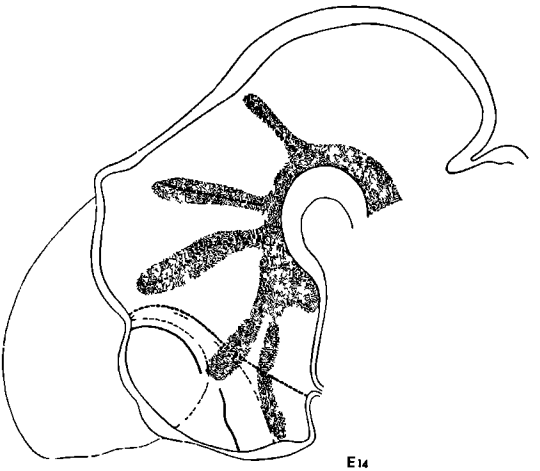


Fig 48 Schematic representation of the zones of early progressive differentiation within the diencephalon.

First the progressive zones will be discussed: From the early progressive tegmentum mesencephali maturation of the neuroepithelium spreads in both the rostral and the dorsal direction. Within the caudal part of the prosencephalon it is observed that the interneuromeric crests possess a somewhat accelerated matrix as compared to the intervening regions. Rostral to the interparencephalic boundary, however, another developmental principle prevails. Here a subthalamic and a hypothalamic cell cord are developing reaching from the tuberculum posterius region to respectively the telodiencephalic border zone and to the optic chiasm. These progressive strips of neuroepithelium form their mantle layer derivatives rather early. Thereby a skeleton is formed of early differentiated zones. This skeleton represents a frame which determines in part what kind of mechanically transforming influences will be exerted by the retarded, more intensely proliferating epithelium.

Let us now turn to a discussion of the relatively retarded regions. As can be seen for instance in fig. 44 they are five in number. The area basal to the hypothalamic cell cord lags far behind when compared to the hypothalamic cell cord itself. The same is true for the region around the sulcus intraencephalicus

anterior; here the preoptic area is the one proliferating most slowly and only reaching matrix exhaustion after the E_{18}-stage. Dorsally in the diencephalon, the pars dorsalis thalami as well as the epithalamus are retarded, but from the E_{16}-stage onwards the pars dorsalis thalami matures at an increased rate, while the epithalamus finally reaches the exhaustion phase after the E_{18}-stage of development. The colliculus inferior, the caudalmost part of the tectum mesencephali, also represents a retarded area, when compared to other mesencephalic structures.

The acceleration of histogenesis in the basal tegmentum mesencephali and in the subthalamic and hypothalamic cell cords, as is found in the Chinese hamster, is in agreement with the factual findings of Kahle. The interpretation which this author presents of his matrix phase maps, is influenced strongly by the assumed presence of longitudinal zones corresponding to the four floors of the His-Herrick-Kuhlenbeck scheme. In addition Kahle discerned a subthalamic zone which is conceived as the rostral continuation of the tegmentum mesencephali and as the dorsalmost part of the hypothalamus. The five floors just mentioned are illustrated in all but the first of his maps and this interferes with an unprejudiced interpretation. Apart from these border-lines, however, our observations concur with Kahle's. As such our results can be considered a confirmation of Kahle's findings. We, however, do not agree with Kahle's interpretation of the pattern observed in the matrix phase maps. In the caudal half of the diencephalon, the accelerated zones are limited in early stages to interneuromeric borders; this holds for the syn-mesencephalic border, for the par-synencephalic border and for the interparencephalic border zone. Rostral to the latter, two accelerated zones dominate the picture, *i.e.*, the subthalamic cell cord and the hypothalamic cell cord, both regions stretching from the tuberculum posterius region forwards and diverging around the optic stalk towards respectively the telodiencephalic border region and the optic chiasm.

When we now attempt to correlate our findings concerning the development of the matrix with those obtained from the study of the ventricular relief (chapter V), the conclusion is reached that both the accentuation and the fading away of sulci are entirely secondary to the histogenetic process. The data obtained in chapter V and chapter VI make it plain that a generally valid description of the development and the nature of the sulci as such cannot be given. Rather it has to be concluded that the developmental history of the ventricular relief can only be understood in relation to the other morphogenetic and histogenetic events of the particular stages in which it is observed.

CHAPTER VII

HISTOGENESIS WITHIN THE DIENCEPHALON: THE MANTLE LAYER

INTRODUCTION

As has been argued in the preceding chapters, the earlier stages of the development of the diencephalon are characterized predominantly by changes in the configuration of the external form, whereas later on the histogenetic processes prevail. This chapter will be devoted to an analysis of the events occurring within the mantle layer. The origin and differentiation of the diencephalic grisea will be described. In addition attention will be paid to the development of the main fibretracts within this region of the brain.

The relations between the processes of matrix proliferation and mantle layer differentiation are very close. Consequently heterochrony phenomena in the activity of the matrix will be mirrored by an equally heterochronous differentiation of the mantle layer peripheral to the matrix. The ensuing description of the differentiation of the mantle layer therefore necessarily complements the analysis of the developing matrix presented in chapter VI. Attention will be paid also to the relationships between mantle layer differentiation and the evolution of the ventricular relief. Prior to the description of our findings, a survey is given of what is known of the process of differentiation within the neuraxis. A summary of the literature pertinent to the development of the grisea in the mammalian diencephalon will be given in a later chapter (IX) in connection with an evaluation of the other developmental aspects of this part of the brain.

The area peripheral to the matrix layer and central to the marginal zone is known as the mantle layer. This area increases in volume as development progresses. In the mature central nervous system it contains all structures typical of the differentiated nervous tissue, that is to say, neurons, glial elements and blood vessels. The blood vessels together with the microglia are mesodermal derivatives; the neurons and the macroglia are, on the contrary, descendants from the neuroepithelial cells. The stages of this descent are not known exactly.

Neuroblast formation

The way in which the inner and outer processes of the neuroblasts loose their original anchorage in the membrana limitans externa and in the ventricular terminal web is a matter of conjecture (Langman *e.a.*, '66; Langman, '68). The

criteria used to define a cell as a neuroblast vary according to the technique employed. A common histological technique, such as the Haematoxyline-Eosine stain, shows hardly more than the nuclei of the cells. The development of the cytoplasmatic extensions of the young neuroblasts cannot be studied with the help of this technique. Typical characteristics of the young neuroblast are the large round shape of its nucleus, the pale nucleoplasm and the darkly staining nucleolus (Langman *e.a.*, '66). The latter appears shortly before the occurrence of Nissl bodies in the perikaryon (Flexner, Flexner and Strauss, '41; Hydén, '43). A silver reduction stain, such as the Cajal technique, permits the demonstration of the early neurofibrils, this being considered the most reliable criterion for identifying a neuron (Cajal, '11; Windle and Baxter, '35; Windle and Austin, '35; Lyser, '66).

Using the Golgi technique, Morest ('69a b, '70) accomplished a differentiation between premigratory and postmigratory neuroblasts. The former is characterized by the presence of a primitive epithelial process, extending towards the ventricular lumen. In addition it possesses axonal and dendritic growth cones. The neuroblast is converted into the postmigratory variety when the epithelial process is lost. The conversion of a neuroblast into a young neuron cannot be observed in the H.E. stained material. Morest ('70) succeeded in the identification of this conversion with the help of the Golgi technique He speaks of neurons when the cells in question acquire the configuration which is characteristic of the region studied. Other useful criteria for identifying neuroblasts are the peripheral position of the cell in the mantle layer and the inability of its nucleus to synthesize DNA as demonstrated by H^3-thymidine labeling experiments.

Glia cell formation

Cajal ('13) defined the astrocyte and Del Rio-Hortega ('19) the oligodendrocyte. The latter coined the term macroglia for the combination of the two types of cells just mentioned, as opposed to the microglia. Kershman ('39) described the origin of the macroglia as ectodermal and that of the microglia as mesodermal. Bailey and Cushing ('25) considered the macroglia and the neurons derivatives from one common stemcell, the bipotential medulloblast, the latter being a relatively undifferentiated precursor of both neurons and macroglial elements located in the matrix layer. Kershman ('38) found no evidence, however, for the existence of these medulloblasts. Instead, he described a so-called subependymal layer that originates in between the matrix and the mantle layer. This subependymal layer consists of neuroglial as well as of neuronal precursors, both capable of division. The existence of this subependymal layer has been confirmed many times for the cerebral hemispheres (Allen, '12; Globus and Kuhlenbeck, '44; Smart, '61; Hinds, '68; Blakemore, '69). Whereas according

to Altmann ('66) this layer plays an important role in the production of glial elements during postnatal life within the telencephalic part of the brain, the differentiation of glioblasts elsewhere in the central nervous system seems to take place in an earlier phase of development, *i.e.,* during the final stages of neuroblast formation (Langman, '68).

These few notes may suffice to show that the debate concerning glia cell formation is far from settled.

Migration of neuroblasts

There is no universal agreement about the mechanism by which the differentiating neuroblasts reach their eventual destination in the mantle layer. Several modes are reported to exist. One way of translocation, derived from studies on H-E stained material, is the so-called radial migration. The cell nuclei migrate simply into a peripheral direction to find their place in the stretch of mantle layer that is bordering on the matrix from where they originate.

Whereas earlier investigators held that neuroblasts migrate as free ameboid elements, Berry and Rogers ('65) arrived at a quite different conclusion. They maintained that it is not the entire cell that migrates, but rather that the nucleus and some of the surrounding cytoplasm moves within the confines of its own primitive offshoots Some years later, Morest ('70) studying a Golgi material of brains of opossum pouch young arrived at a similar conclusion; he did not observe migration in the usual sense of a free, ameboid movement of an entire cell.

The radial migration as observed in the telencephalon by the authors just mentioned, is but one of several possible types of migration. Cells are described in various regions of the brain which migrate in a direction parallel to the external surface (His, '04; Essick, '07; Cooper, '46; Rüdeberg, '61), and the term "tangential migration" is suggested for this mode of migration. "Migration en groupe" is the term used by Hamburger and Levi-Montalcini ('50) to indicate the displacement of grisea 'en masse' through the surrounding nervous tissue. If — and this remains to be proved — Morest's ('70, p. 266) recent dictum, that migration "may only be apparent, in as much as the perikaryon can travel within the elongated neuroblasts", may be made into a generalization to encompass all types of migration, it will be essential for an understanding of the histogenesis of the brain regions which develop by non-radial migration, to explain the growth and direction of the primitive neuroepithelial extensions that permit the migration of the perikarya.

The origin of grisea

Since differentiation is considered to be complementary to matrix cell proliferation it can readily be concluded that heterochrony will be a prominent

feature in mantle layer differentiation. This temporo-spatial patterning characterizes each particular part of the wall of the neural tube by a specific time schedule as to matrix and mantle layer development. Besides, differences may exist in the continuity of the migration process; continuous progress of migration will result in a gradual thickening of the mantle layer whereas a discontinuous "wave-like" migration results in the stratification of the migrated cells into concentric layers (Källén, '54; Bergquist, '57). Moreover the ingrowth of fibre fascicles accompanying the differentiation phenomenon in the literature is sometimes incriminated as the possible cause of the subdivision of the migration layers into parts (Källén, '51; Morest, '70). The stratification of the embryonic and adult nervous system was systematically studied by Senn ('68, '70) in reptiles. Departing from the architecture of the tectum mesencephali as described by P. Ramon (1897), Senn was able to demonstrate in the reptilian brain a consistent stratification of alternating cell and fibre layers in both diencephalon and mesencephalon. It seems probable therefore that, apart from the early local processes of neuromerism, matrix proliferation and early differentiation, the later stages of differentiation may demonstrate a rather different developmental process. In these stages the early maturing (progressive) brain regions may be capable by means of their axonal outgrowths, of exerting a long distance influence upon the conservative (retarded) migration areas.

Most studies on the ontogenesis of the mammalian diencephalon (Bianchi, '09; Droogleever Fortuyn, '12; Miura, '33; Gilbert, '35; J. E. Rose, '42) were carried out in a time when the present views on the dynamics of the neuroepithelial cell population and on the subsequent differentiating process as summarized above were not yet available. In order to arrive at a subdivision of the diencephalon, these authors employed several different criteria, such as the presence of cell condensations, fibre laminae and ventricular sulci. Some authors clearly indicate which criteria they used, but many others rely on the microscopical picture as self-evident. It goes without saying, however, that they do use cytoarchitectonic and myeloarchitectonic features in order to obtain a delimitation.

Within the embryonic brain the grisea become apparent by a process that has been compared with deflocculation in a colloidal system (Miura, '33; Kuhlenbeck, '37). This, however, has nothing to do with an actual movement of cell bodies towards one another. The impression of 'condensing' is obtained by the growth of fibre tracts that surround the grisea; in this way the nuclei which constitute a particular griseum, seem to acquire a more distinct shape. During later development the expanding neuropil within the grisea, consisting of dendritic trees, axonal processes and terminations, quite often causes a fading of boundaries which are quite distinct in earlier phases. J. E. Rose ('42) in his description of the development of the rabbit's diencephalon, distinguishes transitional embryonic areas which result from the splitting of the mantle layer into

several areas. These transitional grisea he calls 'pronuclei', structures which are considered to differentiate later on into definitive nuclear groups.

A number of authors characterize nuclei using cytoarchitectonic criteria. The subjective character of this approach is illustrated by the fact that the cytoarchitectonists are frequently subdivided into the categories of 'lumpers' and 'splitters' according to the fineness of subdivision the authors prefer.

In the present study we do not intend to present a detailed cytoarchitectonic analysis of the diencephalon. In the delimitation of cell masses our main criteria were formed by (1) presence of clear boundary-like and optically empty zones, and (2) differences in the density of neurons. In this chapter the results are presented of an analysis of the structures located in the embryonic mantle layer structures. Of course, it was unavoidable that some of the conclusions, arrived at are indicated already by the terminology employed. This semantic difficulty is to some extent inherent in any presentation of results of embryological investigations.

In studying the brain of the adult Chinese hamster, the existing atlasses and descriptions of the brain of readily comparable mammals such as the mouse (Holmes, '53; Kovac and Dunk, '68), the rat (Gurdjian, '27; Krieg, '32, '44; Zeman and Innes, '63 König and Klippel, '63), and also the Guinea pig (Hess, '55; Völker and Graef, '67; Graef and Völker, '68), the golden hamster (Knigge *e.a.*, '68), and the rabbit (M. Rose, '35; Sawyer *e.a.*, '54) were of great help. The differences between the Chinese hamster brain and that of the mouse and the rat appeared to be morphologically of minor importance.

OBSERVATIONS

Description of mantle layer development in subsequent stages

The development of the central nervous system proceeds as one continuous process, and therefore any subdivision of this process in phases will be of a somewhat arbitrary nature. Mantle layer development was studied in the series mentioned in the list of material (table II, p. 8). In a pilot study the E_{14}-stage and the E_{18}-stage appeared to hold key positions for a proper understanding of the development. The E_{14}-stage retains many characteristics typical of the neuromeric pattern that dominates in the pre-E_{14} period. Yet, at this E_{14}-stage the mantle layer has developed in a number of regions to such extent, that, by means of retrograde studies, it is possible to trace adult regions back to this E_{14}-stage. From this stage on, differentiation continues until at the E_{18}-stage all components of the adult diencephalon can be recognized. At the E_{18}-stage the main morphogenetic changes have come to an end, which makes this stage readily comparable to the adult configuration. In order to arrive at an appropriate description of our observations the delimitable structures within the

mantle layer at each stage were analysed with the help of graphic and three-dimensional reconstructions. From the reconstructions that were prepared three are shown in this chapter (E_{14}, fig. 51; E_{16}, fig. 54; E_{18}, fig. 57). Thus we arrived at a subdivision of our description into the following seven phases; the pre-E_{14} period, the E_{14}-stage, the E_{15}-stage, the E_{16}-stage, the E_{17}-stage, the E_{18}-stage and the post-E_{18} period. They are all, of course, instantaneous pictures of one continuous process, yet together they give a representative portrait of this development.

Mantle layer development before E_{14}

In E_{12} the origin of the secondary neuromeres becomes distinct. As was set forth in the chapter on matrix activity, local differences in development of the neuroepithelium are present, but the formation of a mantle layer can not be ascertained as yet at these stages. The basal parts of the synencephalon and the adjacent area caudal to the eye stalk are more advanced than the remainder of the diencephalon. The neuromeric borders at this stage show up as ventricular eminences corresponding to external constrictions; the position of the cell nuclei of these borders shows an irregularity and crowding, thus making the impression of a kind of septa extending throughout the wall of the neural tube. A different rate of development of the matrix, as compared to the epithelium of the neuromeric bulges, can not be seen at the interneuromeric borders at this stage.

At the E_{13}-stage neuromerism is at its height. Development of a mantle layer can be observed in the basal half of the synencephalon and in an area reaching from there up to the basal border of the optic stalk. In addition one can observe that the septum-like interneuromeric borders show a progressive development in their basalmost parts, where they make contact with the progressive basal parts in syn- and parencephalon just mentioned. This applies not only to the syn-mesencephalic border, and the syn-parencephalic border, but also to the interparencephalic boundary which becomes clearly visible only now. In the more rostral parts of the prosencephalon no such direct correlation can be observed between neuromerism and mantle layer development. Here the developing mantle layer instead of emphasizing the neuromeric pattern tends to obscure it altogether. Due to the development of the so-called hypothalamic cell cord (Gilbert, '35), the border between the parencephalon anterius and the postoptic neuromere fades away. More dorsally this border becomes blurred by the development of a progressive area that ranges from the basal synencephalon to the telodiencephalic border zone and by which the parencephalon anterius is divided into two halves. The mantle layer in this region contains a rather compact 'stream' of cells; the direction of this 'stream' is indicated by the orientation of the cellular nuclei, which, lying parallel to the external surface,

point in the direction of the hemisphere stem. The origin of the cells (*i.e.*, the matrix from which they originate) remains obscure. The 'cell-stream' just mentioned was called the subthalamic cell cord by us. This subthalamic cell cord can be followed up to the bottom of the external telodiencephalic groove (s. sulcus haemisphaericus), where a mantle layer consequently is formed. It is not possible to discern any cell condensations at this stage of development that could be interpreted as pronuclei or definitive grisea.

Mantle layer development at E_{14}

Reference to figures 39, 40 and 41 shows that in the E_{14}-stage a mantle layer is present in large parts of the diencephalon and that, where present, this mantle layer shows local differences in width. Cell condensations can be observed within this mantle layer in a number of places; they are represented in figure 51. The mantle layer condensations fall into two categories, *i.e.*, (a) those that can clearly be correlated to neuromerism, and (b) those that do not show this relationship.

It was observed that in the diencephalic regions caudal to the interparencephalic border the neuromeric pattern is stressed by the phenomena of mantle layer differentiation. Rostral to the interparencephalic border mantle layer structures develop without obvious relations to the interneuromeric borders.

The relationships in the part of the diencephalon caudal to the interparencephalic border will be discussed first. Here three interneuromeric borders can be discerned (*i.e.*, the syn-mesencephalic border, the syn-parencephalic border and the interparencephalic border, cf. fig. 49), which show a distinct relationship with fibre tracts. In the syn-mesencephalic border the commissura posterior is seen to develop (fig. 50, 51); at the junction between synencephalon and parencephalon posterius the fasciculus retrollexus is observed. Within the interparencephalic border in this E_{14}-stage a number of fibres are observed, running the length of this boundary in a basodorsal direction; Gilbert ('35) described this bundle as the "tractus of the zona limitans" (fig. 51).

Surveying mantle layer differentiation in this part of the diencephalon in the stage under consideration (E_{14}) a basodorsal gradient can be observed. Within the progressive basal parts, which are located rostral to the mesencephalic tegmentum, two areas can be observed: (1) In the basis of the synencephalon the prerubral tegmentum contains a pronucleus foreshadowing the nuclei of Cajal and Darkschewitsch (fig. 50d, 51). (2) The prerubral tegmentum merges without any clear-cut border with the more rostrally situated regio subthalamica. The latter forms the basal part of the parencephalon posterius and extends into the basis of the parencephalon anterius. At the junction of the two regions the tegmental cell cord (Kuhlenbeck, '54) can be discerned (fig. 50d, 51).
From these basal regions differentiation apparently spreads in a dorsal direction.

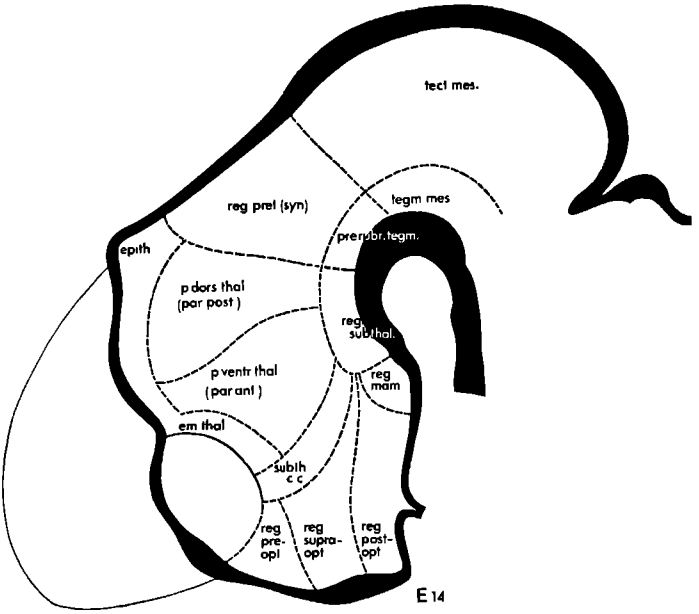


Fig 49 Schematic representation of ontogenetic units within the E_{14} Chinese hamster diencephalon.

The interneuromeric borders take the lead in this process. Thus in this E_{14}-stage within the dorsal part of the synencephalon the future pars praecommissuralis pretecti appears as a pronucleus, flanking the fasciculus retroflexus caudally (cf. fig. 50b,c, 51).

Within the epithalamic part of the parencephalon posterius a primordial lateral habenular nucleus can be recognized. A retarded area making up the largest part of the parencephalon posterius prefigures the pars dorsalis thalami.

Within the area located rostral to the interparencephalic border, the mantle layer develops in such a fashion that the anterior parencephalon falls apart into a number of areas while the interneuromeric borders become unrecognizable and fade away (see fig. 49). In this process two leading factors are demonstrable.

Firstly, in the mantle layer several cell condensations develop that do not show any relationship to neuromerism. In the caudalmost part of the parencephalon anterius the same cell condensation is observed that was described in the E_{13}-stage. It consists of cells arranged parallel to the external surface, and the cell nuclei are pointing with their long axis towards the basis of the telencephalic evagination. This condensation was called in E_{13} the subthalamic cell cord. The basal border of this cell condensation is slightly more developed (cf. fig. 51, subth.c.c.). A second mantle layer condensation extends from the tegmental part of the diencephalon into the primordium of the chiasma opticum. This is the hypothalamic cell cord of Gilbert ('35). Within this hypothalamic cell cord

two grisea could be discerned (fig. 51). These are the anlagen of the nucleus dorsomedialis hypothalami and of the nucleus ventromedialis hypothalami. The hypothalamic cell cord thus forms the dorsal boundary of an area consisting of the postoptic neuromere and the basal part of the parencephalon anterius, which behaves as a morphogenetic unit during further development.

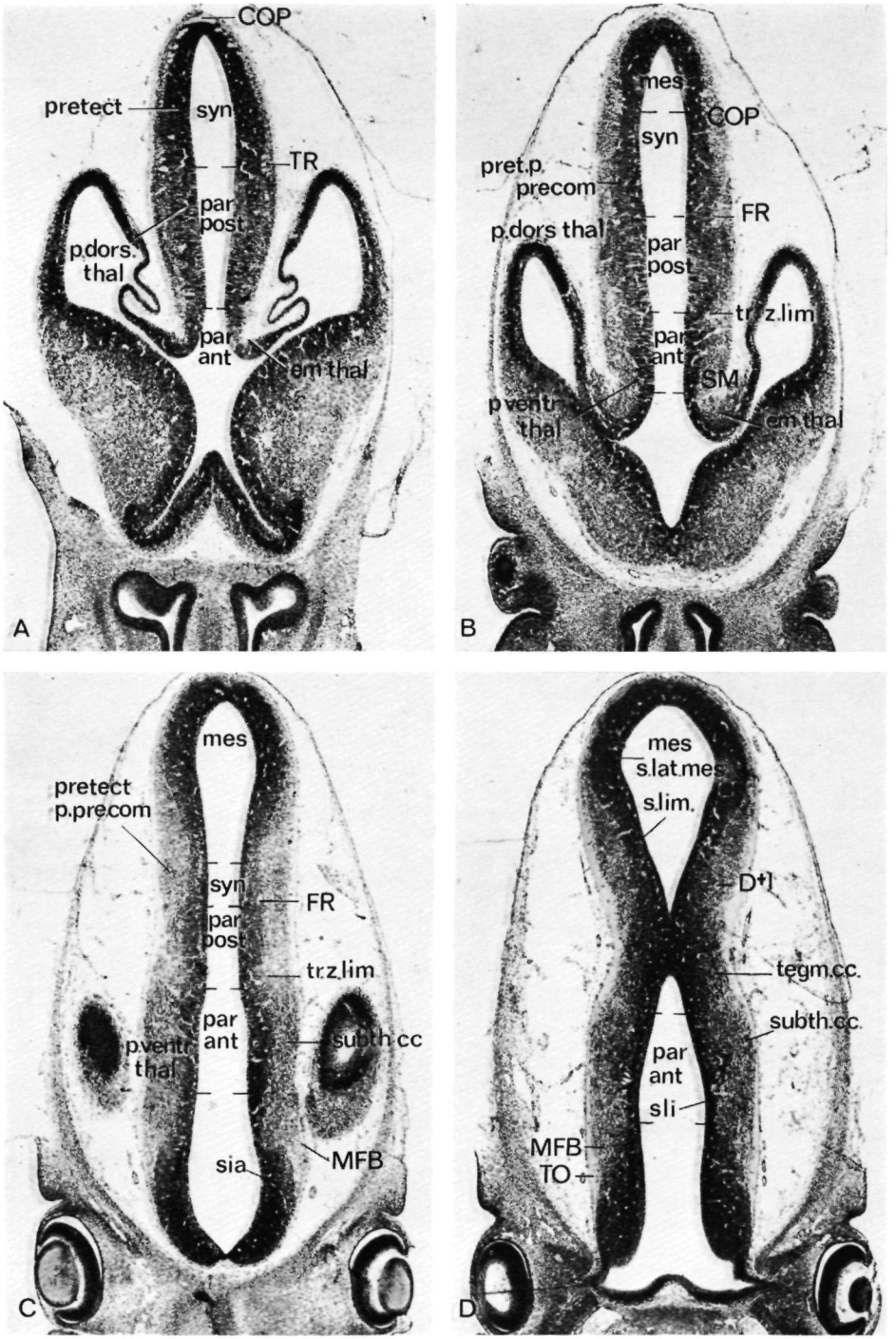
A second factor which obscures the original neuromeric pattern in the region rostral to the interparencephalic border is the development of fibre bundles. In the reconstruction represented in fig. 51 the stria medullaris (extending between the basal telencephalic region and the habenular anlage), the tractus opticus and the fasciculus mamillotegmentalis are depicted. Not incorporated in the figure is the medial forebrain bundle that can be discerned at this stage curving with a basally directed convexity towards the tegmentum. The cells of the area hypothalamica lateralis are observed, intermingled with the fibres of this bundle.

In the rostralmost part of the diencephalon, then, several areas can be discerned which do not show a direct relationship to the original neuromeric pattern, but which behave as ontogenetic units during further development and can be traced from this E_{14}-stage up to the adult configuration (see fig. 49). Immediately rostral to the interparencephalic border the pars ventralis thalami and the regio subthalamica are located. The former is delimited dorsorostrally by the eminentia thalami, which at this stage contains a nucleus accompanying the stria medullaris (nucleus taeniae; cf. fig. 51). The regio subthalamica is, without a clear delimitation, continuous with the basal part of the parencephalon posterius and contains the cell 'stream' (fig. 51; subth.c.c.) described above. The regio mamillaris is characterized by the presence of the mamillotegmental fascicle. Rostral to it the regio postoptica extends from the regio mamillaris towards the optic chiasm. Its dorsal border is formed by the hypothalamic cell cord and in its basis the infundibulum is present. The region located dorsorostral to the hypothalamic cell cord and caudal to the sulcus intraencephalicus anterior was labeled the regio supraoptica. Rostral to the sulcus just mentioned the regio preoptica reaches up to the torus transversus.

Summarizing it can be stated that this E_{14}-stage forms a crucial stage in the study of mantle layer development, because on the one hand certain features of neuromerism can still be discerned, whereas on the other hand many features of the adult configuration are foreshadowed. Anticipating our later conclusions we should like to emphasize already at this juncture that remnants of neuromerism remain recognizable throughout the development of the diencephalon, namely in its caudal part.

Mantle layer development at E_{15}

Considerable changes have occurred in the mantle layer and the E_{15}-stage has already more features in common with the E_{16} than with the E_{14}-stage.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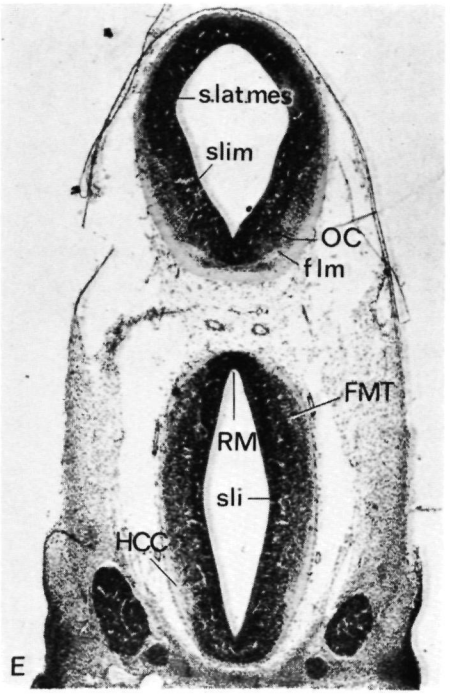


Fig. 50 Transverse sections through the E_{14} diencephalon. The direction of sectioning and the level of the sections is indicated in the diagram.

As can be concluded from a comparison between the matrix phase maps of E_{14} (figs. 39, 40, 41) and those of E_{15} (figs. 42 and 43), the region characterized by the presence of a mantle layer has increased in the E_{15}-stage.

For the sake of clarity we decided to divide the diencephalon into two divisions, a caudal part which is characterized by neuromerism and a rostral non-neuromeroid region.

Within the region caudal to the parencephalon anterius the interneuromeric borders that have been observed in the preceding phase of development can still be discerned. They are most distinct in the dorsal part of the brain tube. Here, the tectum mesencephali, the pretectal part of the synencephalon, the pars dorsalis thalami, and the pars ventralis thalami are separated by the commissura posterior, the fasciculus retroflexus, and the zona limitans intrathalamica, respectively.

In the basal part of the brain tube caudal to the parencephalon anterius, the mesencephalic tegmentum continues rostrally in the synencephalon as the prerubral tegmentum; rostral to the syn-parencephalic border, the regio subthalamica forms a common basis for both the parencephalon posterius and part of the parencephalon anterius.

Within the areas enumerated above, mantle layer development has progressed to some extent. First the early differentiating basal part of the caudal diencephalon will be described. Next the dorsal regions will receive attention; in general, they are retarded when compared to the basal areas.

The tegmentum mesencephali contains the oculomotor nucleus; the nucleus ruber and substantia nigra have not yet been delimited with certainty. The pronucleus that was observed within the prerubral tegmentum (*i.e.*, the basal part of the synencephalon) in the E_{14}-stage, has segregated in the nucleus interstitialis of Cajal and the nucleus of Darkschewitsch. A rostral continuation of the longitudinal cell column that gives origin to the two nuclei just named extends into the regio subthalamica (rostral to the fasciculus retroflexus bundles) as the tegmental cell cord. Within the regio subthalamica the supramamillary commissure develops, which crosses the midline. On transverse sections the mantle layer shows here a reticulated appearance. Rostrally the subthalamic region passes gradually into a relatively undifferentiated area. Since the subthalamic region forms the earliest differentiating part of the prosencephalon, it is an attractive hypothesis to postulate in this region an organizer centre for the rostralmost parts of the brain.

Within the dorsal divisions of the caudal diencephalic neuromeres, mantle layer development also has progressed. The enormous increase in volume of the commissura posterior has caused the subdivision of the regio pretectalis into a caudal commissural and a rostral precommissural part. On horizontal sections the grisea developing from the precommissural part can be seen, extending in

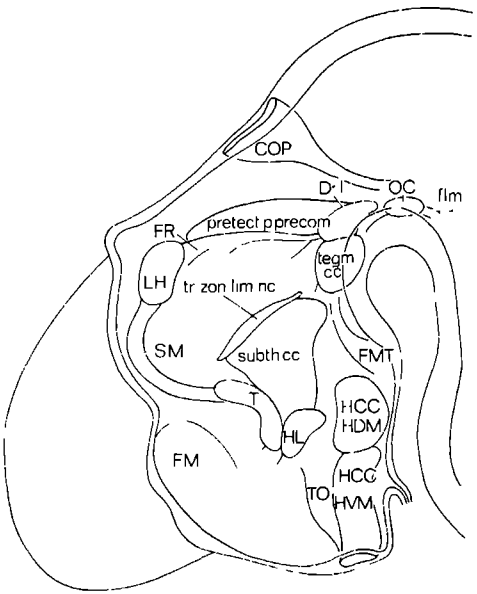


Fig 51 Reconstruction of mantle layer structures in the E_{11} diencephalon

a caudolateral direction; in this way they partly find a place lateral to the posterior commissure.

The fasciculus retroflexus, which marks the syn-parencephalic border, by now is seen extending to the top of the plica encephali ventralis.

The primordium of the habenular nuclei is observed in the dorsocaudal part of the parencephalon posterius. When compared to the preceding stage it is enlarged considerably. Rostrally this epithalamic part of the parencephalon posterius narrows to a slender column of cells extending towards the velum transversum. The stria medullaris is seen in a superficial position at the junction of the differentiated epithalamus and the roof plate; the latter structure shows a primitive epithelial aspect.

The pars dorsalis thalami has increased its transversal dimension just like the other parts of the wall of the brain tube. The boundary planes that have originated from this increase in width are characterized by a rostrally convex shape, not unlike the curved course shown by the dorsal part of the syn-mesencephalic boundary plane. Therefore, the line of intersection between these boundary planes and the external surface of the brain tube is found at a considerably more caudal level than the line of intersection with the ventricular surface. The caudal boundary, marked by the fasciculus retroflexus, extends from this bundle laterally; here a kind of septum consisting of radially oriented cells is observed. The rostral boundary is formed by the zona limitans intrathalamica and by a tract which is located lateral in this zone. The pars dorsalis thalami itself continues to show a very simple structure in E_{15}. Medially an active matrix is present and the part that lines the ventricle shows a strongly basophilic appearance. The lateral mantle layer has a rather loose aspect which is caused by the presence of the first ingrowing fibres of the radiatio thalamostriatalis. In between the medial and lateral zones just mentioned, a light zone is observed which was erroneously called zona limitans intrathalamica by J. E. Rose ('42) [6]).

The primordial pars dorsalis thalami is seen to extend rather far caudally. Since the basal border of the pars dorsalis thalami, in a relative sense, becomes increasingly small, the impression is created that this formation tapers to a caudal end, where the external caudal and rostral border-lines meet.

We will now turn to a description of the structures rostral to the interparencephalic border. In E_{14} we came already to the conclusion that there was a fading of neuromeric borders in this part of the brain. The early mantle layer differen-

6 The term zona limitans intrathalamica is universally used to indicate the fibre layer developing within the interparencephalic boundary, which later on forms the lamina medullaris externa. The structure within the pars dorsalis thalami indicated by Rose (42) by the same name has a relationship to the definitive lamina medullaris interna Here confusion is imminent and all the more so as in the earlier literature (Droogleever Fortuyn, '12, Kuhlenbeck, '30) the pars ventralis thalami of our denomination was thought to differentiate into the eventual ventrobasal complex of the dorsal thalamus

tiation in this region was described as consisting of two columns of differentiated cells, extending from the regio subthalamica to the telodiencephalic border-zone (*i.e.*, the subthalamic cell cord) and to the developing chiasma opticum (*i.e.*, the hypothalamic cell cord), respectively.

Within the dorsal part of the parencephalon anterius, a primordial pars ventralis thalami can be delimited from the more basally situated subthalamic cell cord. The latter, which is clearly a forward extension of the regio subthalamica, develops into the nucleus entopeduncularis. Dorsorostral to the parencephalon anterius the eminentia thalami can be distinguished. In our opinion it was not possible to interpret this structure with certainty as either a part of the parencephalon anterius or of the optic neuromere. In the most lateral parts of the eminentia thalami the stria medullaris curves, in a very superficial position, towards the basis telencephali.

In connection with the stria medullaris a cell mass is observed that presumably corresponds to the nucleus ovalis of Bellonci as depicted by Gilbert ('35) in the human embryo. It is very probable that at this stage there is a continuity of this cell mass with the cells intermingled with the fibres of the tract of the zona limitans.

Within the basal part of the parencephalon anterius, the matrix located basal to the sulcus diencephalicus ventralis and dorsal to the fasciculus mamillotegmentalis gives origin to the suprapeduncular complex (Rose, '35). This complex consists of a caudal and a rostral part: The caudal part contributes to the development of the partes ventrales of the corpus geniculatum mediale and laterale and to the cells intermingled with the tract of the zona limitans. The rostral part of the suprapeduncular complex contributes to the future zona incerta.

The primordial corpus subthalamicum Luysi is observed at this stage. It develops from a tangentially migrating 'stream' of cells that, originating from the matrix of the supramamillary recess, gradually shifts in a rostrodorsal direction.

In the region rostral to the parencephalon anterius the only important change which has occurred is the development of the medial forebrain bundle. This structure gives the hypothalamic region a common denominator, but the ventricularly located regions that were described in the E_{14}-stage, can still be recognized with great ease. Within the border-zone between the regio supraoptica and the regio postoptica a supraoptic commissural system is seen to develop.

Mantle layer development at E_{16}

A comparison between the E_{15}-matrix phase map (fig. 42) and those of the E_{16}-stage (figs. 44, 45), shows a further increase of the area in which a mantle layer is present. The regions described in the preceding stages still can be discerned as is shown in figure 52. This means that in the E_{16} embryo neuromeric

borders can still be observed in the part of the brain caudal to the interparencephalic border, but that more rostrally the organization of the brain is entirely dominated by the secondary ontogenetic units that were already described for the E_{14}-stage.

The neuromeric pattern caudal to the parencephalon anterius is complicated by the development of subregions. In the dorsal syncncephalon (the regio pretectalis) a caudal commissural part and a rostral precommissural part are observed. Within the parencephalon posterius the epithalamus and pars dorsalis thalami develop. Rostral to the interparencephalic boundary the pars ventralis thalami can be distinguished. The basal portions of these caudal diencephalic neuromeres are less easily identified. Clear boundary lines are identifiable only in favourable sections.

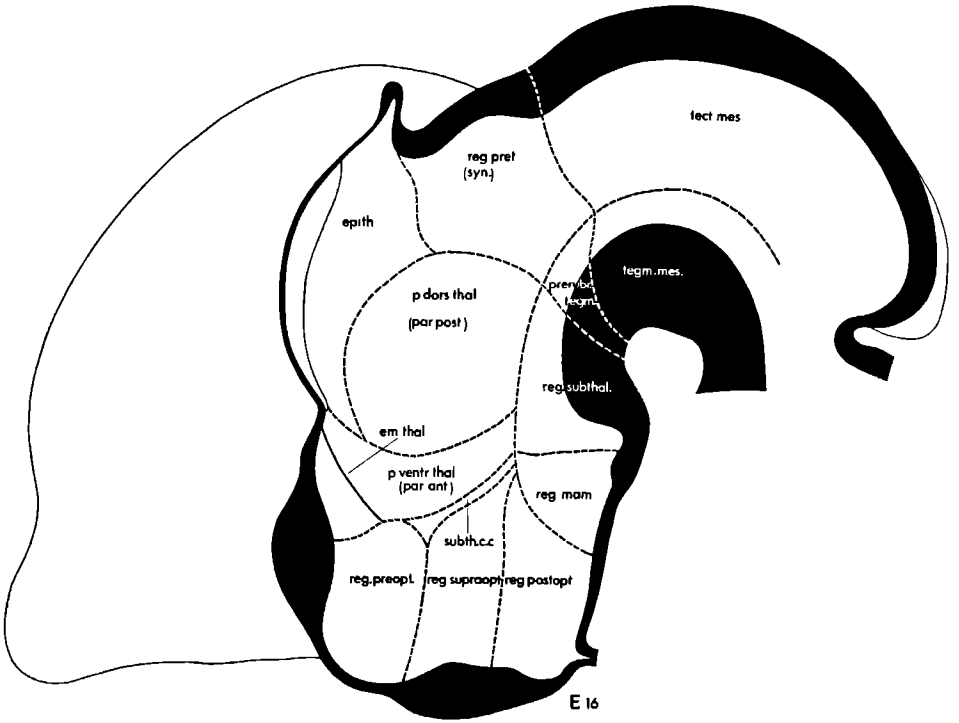


Fig. 52 Schematic representation of ontogenetic units within the E_{16} Chinese hamster diencephalon.

Rostral to the interparencephalic border the regio preoptica, regio supraoptica, regio postoptica and regio mamillaris can be discerned. As a consequence of the expansive growth of the epithalamic and dorsal thalamic regions the regions of the pars ventralis thalami and of the subthalamic cell cord have relatively decreased.

The areas enumerated above will be described with the help of a series of

transverse sections (figs. 53a-d) and with reference to fig. 54, which presents a reconstruction of the E_{16} mantle layer configuration.

In order to facilitate the orientation, the interneuromeric borders and the fibre tracts will be discussed first and only subsequently attention will be paid to the grisea that can be discerned at this stage of development.

The neuromeric border between mesencephalon and synencephalon is marked in its dorsal part by the development of the posterior commissure; basally this border runs just in front of the nucleus ruber that now has become recognizable.

The border between synencephalon and parencephalon posterius is characterized by the fasciculus retroflexus and by the so-called posterior thalamic septum (Coggeshall, '64).

The rostral border of the parencephalon posterius is marked by the zona limitans intrathalamica. The tract of the zona limitans cannot be discerned any more; instead a dorsal continuation of the pars geniculata of the suprapeduncular complex can be recognized, which is situated at the external margin of the zona limitans intrathalamica. This structure clearly has developed from the interstitial nucleus described in E_{15} (cf. figs. 53b-c, fig. 54).

The border between the parencephalon anterius and the optic neuromere has faded entirely. It could not be determined to which of these two neuromeres the eminentia thalami belongs.

In fig. 54 the fasciculus mamillotegmentalis can be observed crossing medial to the fasciculus retroflexus on its way to the midbrain. The optic chiasm can be observed and the optic tract extends from there to the pars dorsalis thalami.

The highly complex situation existing in the telodiencephalic border-zone could only be unravelled by the combination of observations derived from series sectioned in various directions. Four major fibre bundles develop in this region, *i.e.*, the capsula interna (at this stage represented by the radiatio thalamostriatalis), the medial forebrain bundle, the stria medullaris and the stria terminalis.

The radiatio thalamostriatalis connects the striatal anlage with the pars dorsalis thalami. It is illustrated in figs. 53a-b. The medial forebrain bundle (figs. 53a-b) also connects telencephalic and diencephalic structures. The stria medullaris shows a typical S-formed course and at the level of the foramen of Monro it lies in a superficial position. Passing external to the radiatio thalamostriatalis and to the medial forebrain bundle, it reaches the basal telencephalic region (fig. 54). In the floor of the foramen of Monro the primordial stria terminalis can be observed riding on the capsula interna anlage on its way from the amygdaloid complex to the preoptic region (fig. 54).

After this discussion of the fibre tracts we will now direct our attention to the grisea that could be distinguished in the E_{16} diencephalon. Most of these cell masses are represented in the reconstruction (fig. 54). In the description we will proceed from caudal to rostral.

In the tegmentum mesencephali the nucleus ruber and the substantia nigra

can be observed. The latter structure originates from a cell 'stream' which migrates from the matrix region near the fovea isthmi in the median plane and splits basally into a left and right half like an upside-down fountainhead. Between the fasciculus retroflexus and the fasciculus mamillo-tegmentalis of both sides the migrating cells reach the basal surface of the brain. The median nucleus interpeduncularis is also thought to originate from this 'stream' of cells.

Within the prerubral tegmentum (basal to the pretectal region) the nucleus interstitialis of Cajal and the nucleus of Darkschewitsch are observed.

The subthalamic region is characterized at this stage by the presence of a well-developed suprapeduncular complex and by the superficially located corpus subthalamicum. It contains in addition the tegmental cell cord that has differentiated into a posterior hypothalamic nucleus (fig. 53c) and a reticulated area through which the fibres of the medial forebrain bundle and those of the striotegmental tract pass to more caudal levels of the brain.

Fig. 54 shows the suprapeduncular complex consisting of a pars geniculata and a pars incerta. The pars geniculata continues dorsally in between the pars ventralis thalami and the pars dorsalis thalami as an interstitial nucleus located at the place of the former tract of the zona limitans. The dorsalmost point of this cell column is located at the place of the nucleus ovalis (Gilbert, '35). The pars incerta of the suprapeduncular complex abuts on the basal border of the pars ventralis thalami. In a rostral extension of the regio subthalamica the nucleus entopeduncularis can be observed.

It has already been pointed out that the pretectal region, the pars dorsalis thalami and the epithalamus constitute the dorsal parts of – still recognizable – neuromere formations.

Two grisea are illustrated within the pretectal division of the diencephalon rostral to the posterior commissure. The dorsal nucleus represents the nucleus pretectalis, the basal one is the nucleus posterior thalami, which thus appears to be a synencephalic derivative.

In the epithalamic region of the parencephalon posterius the radiation of the fasciculus retroflexus can be observed. A nucleus habenularis lateralis is also present. The stria medullaris while ascending in the epithalamic zone, gradually decreases in thickness and eventually reaches the midline in the habenular commissure. The rostral border of the pars dorsalis thalami (towards the parence-

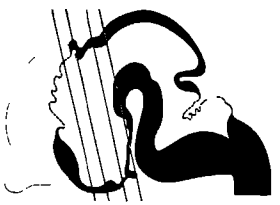
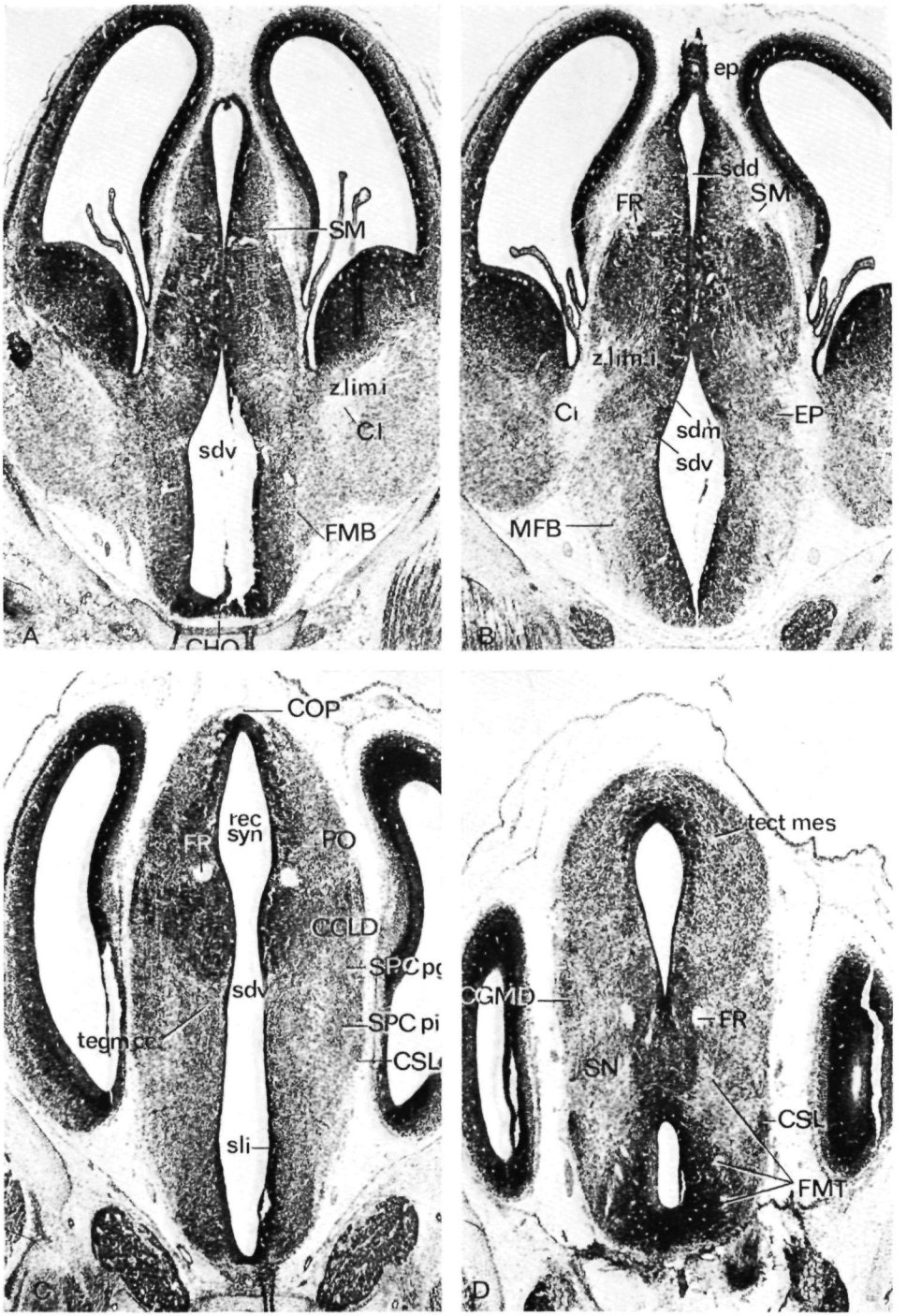


Fig. 53 Transverse sections through the E_{16} diencephalon. The direction of sectioning and the level of the sections is indicated in the diagram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phalon anterius) is characterized by a rostral convexity. Since the pars dorsalis thalami is depicted as translucent in fig. 54, the structures participating in the formation of its 'bed' can be clearly distinguished. The thalamic bed thus illustrated consists almost exclusively of derivatives from the dorsal part of the parencephalon anterius. Only laterally the interstitial nucleus of the tract of the zona limitans is observed as a derivative from the pars geniculata of the suprapeduncular complex. Medial to this structure the cells of the primordial nucleus reticularis thalami are interposed between the fibres of the radiatio thalami.

The mediodorsal part of the zona incerta appears to be a derivative from the pars ventralis thalami. Dorsally the eminentia thalami is continuous with the pars ventralis thalami: no clear boundary can be indicated between these two parts of the diencephalon. From this region the pars superior massae cellularis reunientis (Miura, '33; Kuhlenbeck, '37) or the suprahypothalamic complex (J. E. Rose, '42) develops.

We will now turn to a description of the regions that together constitute the hypothalamus, *i.e.*, the regio mamillaris, the regio postoptica, the regio supraoptica and the regio preoptica (fig. 54).

The mamillary region is characterized by the presence of mamillotegmental fibres and no specific cellular condensations can be observed here. In a superficial position to the mamillary primordia, cells can be observed that migrate tangentially, comparable to the way the corpus subthalamicum Luysi originates.

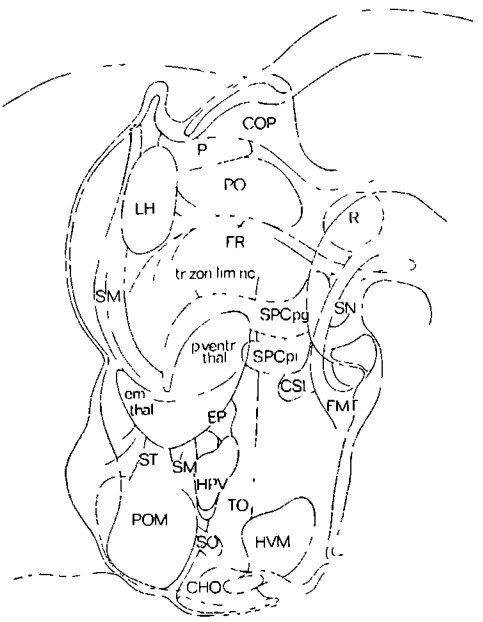


Fig 54 Reconstruction of mantle layer structures in the E_{16} diencephalon, the pars dorsalis thalami is depicted as translucent.

The sulcus lateralis infundibuli marks the rostral boundary of the mamillary region. This border, however, becomes increasingly vague when it is followed in a dorsal direction.

A cellular condensation is observed within the hypothalamic cell cord, which is situated in the regio postoptica, rostral to the regio mamillaris. This is the nucleus principalis of Cajal or the nucleus ventromedialis of more recent authors. Dorsally the boundary between mamillary and postoptic regions fades and the two regions here fuse to form a common area dorsalis hypothalami.

The supraoptic decussations and an early differentiated area rostral to the postoptic region mark the boundary with the regio supraoptica. Two nuclei of this region are represented in the reconstruction; the nucleus paraventricularis and the nucleus supraopticus. The former occupies a mediodorsal position; the nucleus supraopticus is placed basolaterally, resting on the optic tract. The medial forebrain bundle passes between the two grisea just mentioned; it contains a diffuse collection of interstitial cells that together form the area lateralis hypothalami. The stria medullaris fibres pass through the lateral part of the area lateralis hypothalami on their way to the basal telencephalon.

The regio preoptica is located rostral to the supraoptic region; it is characterized by the radiating stria terminalis fibres and their interstitial cells. The stria terminalis forms a continuous band riding over the internal capsule from the amygdala to the preoptic medial parts that are represented in the reconstruction (fig. 54).

Mantle layer development at E_{17}

Comparison of the E_{17}-stage (fig. 46) with figures 44 and 45 (E_{16}) on the one hand and with figure 47 (E_{18}) on the other, shows that the E_{17}-stage takes a fairly intermediate position as far as the area occupied by mantle layer structures is concerned. Therefore this stage will not be described *in extenso*.

Remarkable features of diencephalic development observed at this stage are: the development of peduncular fibres peripheral to the corpus subthalamicum Luysi and the differentiation of two grisea surrounding the nucleus ventromedialis hypothalami at its caudal and basal sides, *i.e.*, the nucleus dorsomedialis hypothalami and the nucleus infundibularis.

Mantle layer development at E_{18}

Mantle layer differentiation has started everywhere in the diencephalon as can be concluded from figure 47. The overall appearance of the brain at this stage shows a more definitive configuration than was the case in the preceding phases of development. Ontogenesis has progressed sufficiently far to allow a reliable comparison with the adult brain. Yet, several embryological landmarks of

earlier stages (as, for instance, the caudal neuromere derived boundaries) have been preserved. Hence, the E_{18}-stage was chosen for the exploration of the ontogenesis of the diencephalon in both the descriptive and the autoradiographic part of this study. At this stage principally the same regions could be discerned as in E_{16}. Within the basal regions rostral to the tegmentum mesencephali, however, a delimitation becomes difficult. For a survey of the regions that could be discerned the reader is referred to figure 55 which, together with figures 49 (E_{14}) and 52 (E_{16}), provides a survey of the transformations that have taken place. This comparison shows that the subdivision of the caudal diencephalon which was derived directly from the neuromeres, has been entirely preserved.

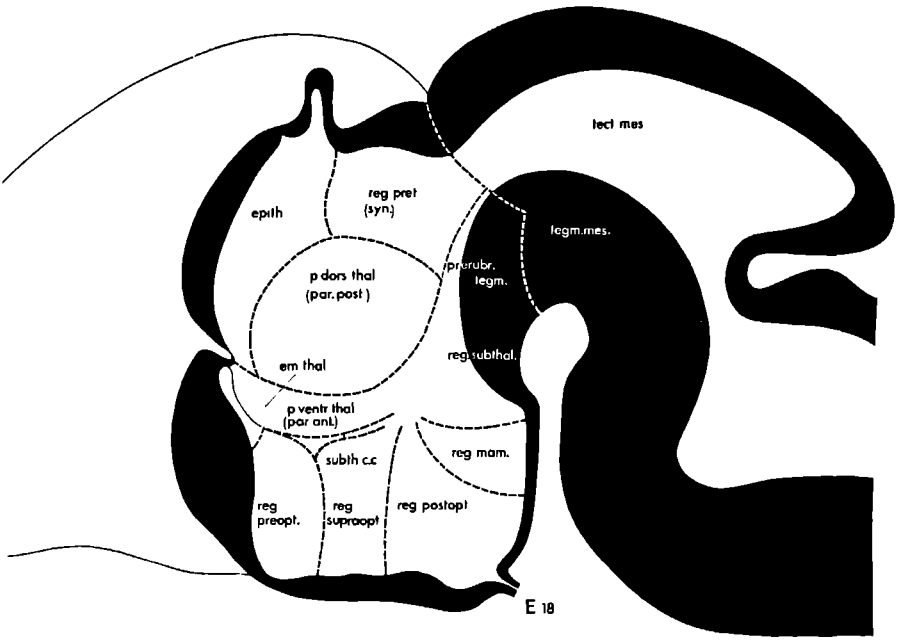


Fig. 55 Schematic representation of ontogenetic units within the E_{18} Chinese hamster diencephalon.

The description of the mantle layer structures will be given with the help of a series of transverse sections (figs. 56a-d) and of a reconstruction (fig. 57). The di-mesencephalic and the di-telencephalic boundaries will be discussed first. Then the intradiencephalic borders and the fibre tracts will receive attention, and, finally, the derivatives of the mantle layer, *i.e.*, the grisea will be described.

The di-mesencephalic or syn-mesencephalic boundary is marked in its dorsal half by the presence of the posterior commissure. Basally it passes rostral to the mesencephalic nucleus ruber and substantia nigra. The tegmental structures seem to have a tendency to descend into the anterior limb of the plica encephali

ventralis. The basal half of the syn-mesencephalic border therefore makes a rostrally obtuse angle with the dorsal half. When the whole of the syn-mesencephalic border-plane is laterally traced from the median plane, it is seen tilting backwards around a transverse axis. This results in a rather complex boundary configuration; dorsally the syn-mesencephalic border-plane is caudally concave, thus embracing the rostral part of the tectum mesencephali. Basally the border-plane is rostrally concave, in this way embracing the basal diencephalic parts. In figure 58 a schematic representation of this boundary-plane is given, which should be compared with fig. 57.

As far as the di-telencephalic boundary is concerned an early differentiating zone could be observed running laterally from the torus transversus towards the base of the foramen of Monro. The boundary-plane that is supposed to extend from this ventricular zone towards the external surface lateral to the preoptic region, is a much debated one and will be considered in more detail in chapter IX.

We will now turn to a description of the intradiencephalic borders and of the fibre tracts. Rostral to the tegmentum mesencephali the prerubral tegmentum and the regio subthalamica can be discerned. The prerubral tegmentum gradually decreases in volume during its development and at this stage it is even difficult to assess its presence in between the tegmentum mesencephali and the regio subthalamica at some places. In these regions the tractus mamillotegmentalis and the fasciculus retroflexus are observed. The latter structure is marking the synparencephalic boundary. Dorsally its fibres fan out and make it possible to delimit the epithalamus against both pretectum and pars dorsalis thalami. The interparencephalic border remains clearly visible as the zona limitans intrathalamica. The optic tract in this stage can be seen extending clearly towards the corpus geniculatum laterale. In the rostral parts of the diencephalon the same configuration of fibre bundles can be observed as was described in the E_{16}-stage. In addition the anterior commissure has now become clearly visible as is illustrated in figure 57.

The grisea that have developed will be discussed in the same sequence as in the description of the grisea in E_{16}. Hence we will first discuss the nuclei discernible in the part of the diencephalon caudal to the parencephalon anterius;

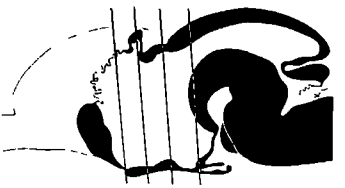
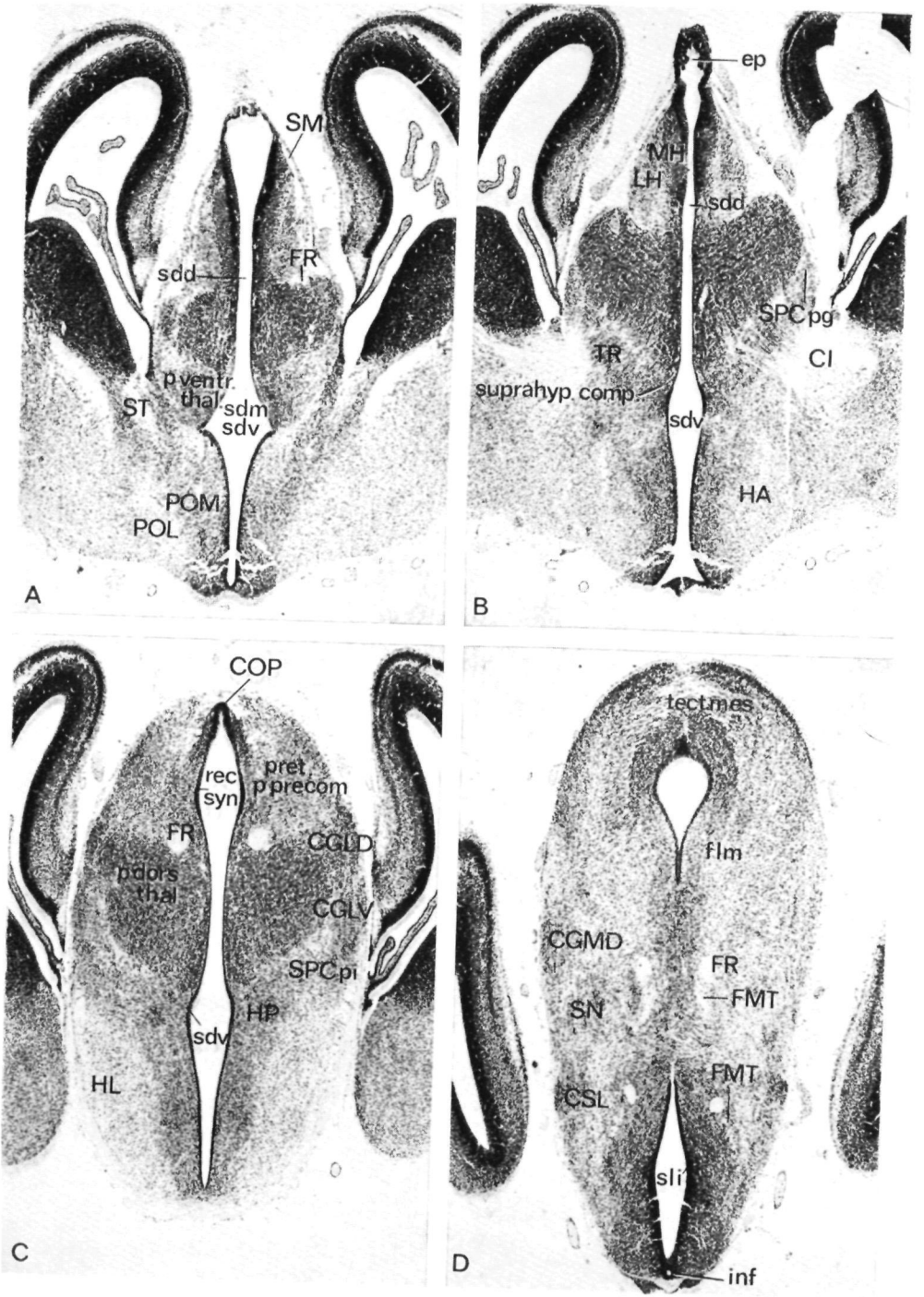


Fig. 56 Transverse sections through the F_{18} diencephalon The direction of sectioning and the level of the sections is indicated in the diagram.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next the structures that occupy a more rostral position will be discussed.

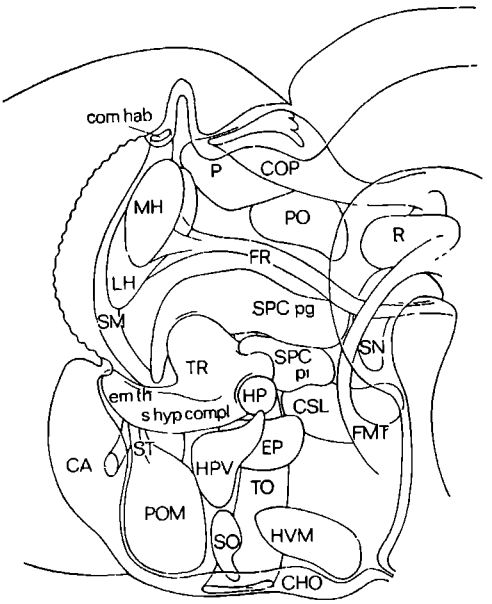
In the basal synencephalon the prerubral tegmentum contains the nuclei of Cajal and Darkschewitsch near the midline. The tegmental cell cord of earlier stages has undergone a dissolution and in its place a reticulated substance is observed through the meshes of which longitudinal fibre bundles take their course. More laterally the prerubral tegmentum looses its identity, as has been described above.

Rostral to the prerubral tegmentum the regio subthalamica is observed. Here the suprapeduncular complex shows the same 'embracing' structure as was described for the basal syn-mesencephalic border-plane. The pars geniculata is located superficially between pars dorsalis thalami and pars ventralis thalami. More rostrally the pars incerta also develops in a superficial position.

The corpus subthalamicum which originates caudobasally to the fasciculus mamillotegmentalis from the matrix of the recessus supramamillaris, is extending rostrodorsally almost up to the level of the optic tract.

The dorsal structures corresponding to the regions described thus far are the regio pretectalis in the synencephalon and the epithalamic and dorsal thalamic regions in the parencephalon posterius.

In the regio pretectalis a commissural part and a precommissural part are discerned. In the commissural part an interstitial nucleus of the posterior commissure and a nucleus centralis subcommissuralis could de identified at this stage. Within the precommissural part a nucleus pretectalis and a nucleus posterior thalami were discerned.



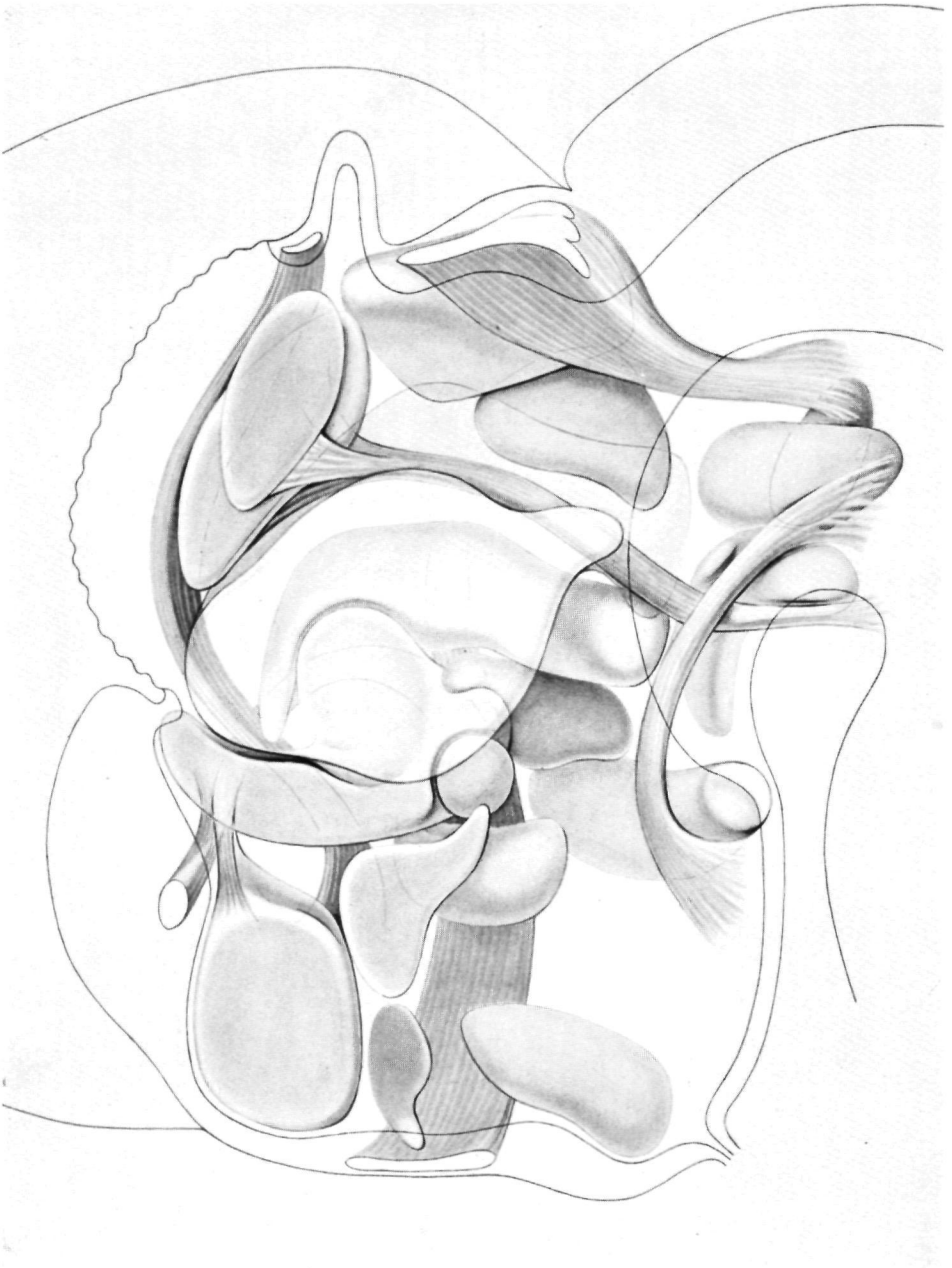


Fig. 57 Reconstruction of mantle layer structures in the E_{18} diencephalon; the pars dorsalis thalami is depicted as translucent.

The epithalamus is still a relatively large region, extending from the velum transversum to the pretectum all along the dorsal border of the parencephalon posterius. Two nuclei could be distinguished in this region, *i.e.*, the early differentiating nucleus habenularis lateralis and the retarded nucleus habenularis medialis.

The two nuclei that can be discerned within the pars dorsalis thalami at this stage are the dorsal parts of the corpus geniculatum mediale and laterale. The corpus geniculatum mediale can be recognized by virtue of its caudobasal position, and the corpus geniculatum laterale is characterized by its connections with the optic tract. As far as the remainder of the pars dorsalis thalami is concerned, a homogeneous and basophilic appearance is observed. No trace of a pronucleus is observed as yet and the internal configuration of this part of the brain remains obscure.

As was mentioned above, the interparencephalic border is characterized by the zona limitans intrathalamica and the laterally placed pars geniculata of the suprapeduncular complex. More medially the bed of the pars dorsalis thalami consists of the nucleus reticularis thalami and, in a juxtaventricular position, of the suprahypothalamic complex. Caudobasal to the latter cell groups a nucleus posterior hypothalami is described: it is characterized by an early differentiation and it is situated just lateral to the sulcus diencephalicus ventralis at this stage.

In a rostrodorsal position to the pars ventralis thalami the eminentia thalami can be observed. Here the epithalamus, pars dorsalis thalami and pars ventralis thalami are continuous and it is our opinion that a sharp delimitation of the three regions is not possible. In the region of the eminentia thalami, which forms part of the caudobasal margin of the foramen of Monro, the stria medullaris, the capsula interna, the medial forebrain bundle and the stria terminalis all pass in a limited space, thus causing an interlacing appearance in transverse sections (cf. Droogleever Fortuyn '57). The rostral extremity of the interstitial nucleus of the tract of the zona limitans – *i.e.*, the extension of the pars geniculata of the suprapeduncular complex – is seen extending into this region, and is here shown at the spot where one would expect the nucleus ovalis of Bellonci (Addens, '38).

Within the hypothalamic part of the diencephalon the ontogenetic units described for earlier stages can be clearly recognized. In the regio mamillaris the medial and lateral mamillary nuclei can be observed. A premamillary nucleus is also distinguished. Within the postoptic region the nucleus dorsomedialis hypothalami and the nucleus infundibularis have developed in a juxtaventricular position. Slightly more laterally the nucleus ventromedialis hypothalami can be observed. A clear boundary between the postoptic region and the supraoptic region is only observed medially in sections in the conventionally horizontal plane. The medial forebrain bundle which passes in a more lateral position does not show any discontinuity at the same level. Within the regio supraoptica a nucleus suprachiasmaticus and a nucleus hypothalamicus anterior can be ob-

served at this stage. The latter two nuclei seem to be caudal continuations of the periventricular and medial preoptic nuclear primordia respectively. In horizontal sections, however, a clear separation between these areas is observed. The medial and periventricular preoptic areas are represented in the reconstruction and can be seen receiving the stria terminalis bundle. The lateral part of the preoptic region forms a rostral continuation of the interstitial cells in the medial forebrain bundle. It is clearly more differentiated than the other constituents of the preoptic region and borders on the diagonal band of Broca.

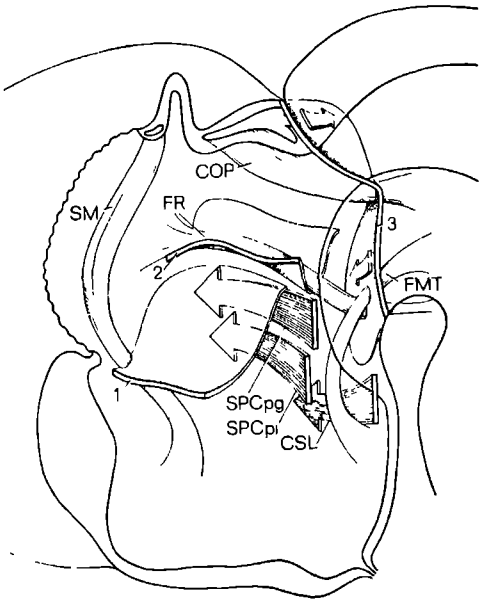


Fig. 58 Schematic representation of the boundary planes between mesencephalon, synencephalon, parencephalon posterius and parencephalon anterius as observed at the E_{18}-stage. The arrows indicate the direction of migration in the corpus subthalamicum Luysi and in the suprapeduncular complex; 1) interparencephalic boundary plane; 2) syn-parencephalic boundary plane; 3) syn-mesencephalic boundary plane.

The period from E_{18} to the adult stage

At E_{18} both the morphogenetic transformations and the histogenetic alterations have progressed to such an extent that the E_{18} diencephalon is readily comparable to the adult configuration. In our study brains of specimens of E_{21}, PN_3, PN_6, PN_9 and PN_{100} (adult) have been included. A description of these stages at this

juncture seems superfluous in view of the objectives of this study. In chapter IX a regional description of the adult brain will be presented and a comparison will be made between the results obtained in our study and the data found in the literature

DISCUSSION AND SUMMARY

The analysis of the development of the mantle layer presented above reveals that the temporo-spatial pattern, which can be discerned in this development is complementary to the patterned way in which the matrix proliferates (cf. chapter VI).

Furthermore, within the diencephalon a caudal and a rostral zone could be clearly differentiated on the basis of differences in the development of the mantle layer; within the caudal part of the diencephalon the neuromeric pattern is stressed by the differentiation process; here the interneuromeric borders acquire a mantle layer structure in which fibre tracts develop. Thus the caudal diencephalic neuromeres can be traced from embryonic stages up to the adult brain. Rostral to the interparencephalic border the neuromeric borders fade away and other, secondary units are formed by the development of differentiating cell cords. In this survey the caudal parts of the diencephalon will be discussed first and next the more rostrally located non-neuromeroid division of the diencephalon will be reviewed.

The tegmentum mesencephali and its rostral continuation into the diencephalon (*i.e.*, the prerubral tegmentum and the regio subthalamica) both show a tendency to differentiate at a relatively early stage. From these basal parts differentiation spreads in a dorsal direction along the interneuromeric crests. It is in these crests that the posterior commissure, the fasciculus retroflexus and the tract of the zona limitans develop. Thus a kind of skeleton is formed which provides some distinct landmarks within the embryonic mantle layer. Tracing these interneuromeric borders revealed that the nucleus posterior thalami and the nucleus pretectalis are of synencephalic origin. The nucleus lateralis posterior on the contrary is a derivative from the parencephalon posterius. A similar ontogenetic analysis of this area in primates will probably yield interesting information as to the obscure provenance of the pulvinar thalami.

The differences manifesting themselves in the dorsal half of this part of the neural tube between the retarded neuromeric centres and the accelerated interneuromeric crests are leveled out in the basal half of the brain. Here, no clearcut borders between tegmentum mesencephali, prerubral tegmentum and the parencephalic regio subthalamica can be indicated at later stages of development. Within the subthalamic region a suprapeduncular complex develops by a migration in lateral direction, originating from a matrix area located dorsal to the

fasciculus mamillotegmentalis and basal to the sulcus diencephalicus ventralis Two parts are discerned within this suprapeduncular complex, the pars geniculata and the pars incerta. The pars geniculata develops into the ventral parts of the corpora geniculata; furthermore it can be followed dorsally in between the pars dorsalis thalami and the pars ventralis thalami. Near the eminentia thalami this column of cells appears to be continuous with a cell mass that bears some resemblance to the nucleus ovalis of Bellonci (as described by Addens, '38). The pars incerta of the suprapeduncular complex forms the caudal part of the definitive zona incerta. The way in which the constituent parts of the suprapeduncular complex and also the corpus subthalamicum develop deserves some comment. Contrary to what is usual in other parts of the diencephalon, in these instances a tangential migration is observed. By this migration an embracing movement is performed, by way of which material is translocated from a medial and basal position into a lateral and dorsal direction. As far as the corpus subthalamicum Luysi is concerned, this type of migration was observed already in 1935 by Gilbert.

Rostral to the interparencephalic border a rather heterogeneous region is observed. When an attempt is made to make a subdivision of the mantle layer of this region, neuromere-derived boundaries are looked for in vain. The first signs of differentiation appear as cell cords extending between the regio subthalamica and the hemisphere stem (subthalamic cell cord) and between the regio subthalamica and the optic chiasm (hypothalamic cell cord). These cell cords play an important role in the subdivision of the rostral part of the diencephalon. The subthalamic cell cord contributes to the formation of the entopeduncular nucleus and probably of the globus pallidus.

The hypothalamic cell cord (Gilbert, '35) can be seen to extend between the regio subthalamica and the chiasma opticum ridge. It divides the basal parts of the diencephalon into two subareas. The area rostral to the hypothalamic cell cord centres around the sulcus intraencephalicus anterior and constitutes two ontogenetic units. Rostral to the sulcus intraencephalicus anterior a preoptic region is located. Caudal to the sulcus intraencephalicus anterior and rostral to the hypothalamic cell cord, a supraoptic region develops in which the paraventricular and supraoptic nuclei originate. The hypothalamic cell cord itself constitutes, together with the region basal to it, the postoptic region, that is to say the infundibular part of the diencephalon. Caudal to this postoptic or infundibular region the mamillary region is the caudalmost constituent of what is classically called the hypothalamus. One of the main characteristics of this mamillary anlage is the presence of mamillotegmental or tegmentomamillary fibre bundles.

Thus, summarizing it may be stated that two characteristics of mantle layer formation prevail in the diencephalon, *i.e.*, the predominance of neuromerism within its caudal part and the development of more or less horizontally oriented cell columns in its rostral part.

In this and the preceding chapters those phenomena have been highlighted which are of importance for a proper understanding of the morphological pattern of the diencephalon. From this understanding of the ontogenetic bauplan a logically consistent subdivision of the diencephalon can be derived. A comprehensive discussion of our findings is postponed to chapter IX where they will be compared with data obtained from the literature.

CHAPTER VIII

HISTOGENESIS WITHIN THE DIENCEPHALON; AUTORADIOGRAPHY

INTRODUCTION

The observations on normal material as reported in chapter VII quite often concern differences in rate of development between particular regions of the brain. To some degree, however, the interpretation of the hematoxyline-eosine stained sections may be subject to the fallacies of subjective judgment.

In order to obtain a material in which the differences in rate of development can be observed as objectively as possible with the techniques available at present, an autoradiographic experiment was designed. This autoradiographic part of our study takes advantage of the fact that, when a labeled precursor of DNA is offered to a proliferating cell population, it will be incorporated premitotically into the nuclei of the dividing cells. Afterwards, this labeled DNA will be transmitted to the offspring of each particular cell.

We applied this principle in our study of the embryonic brain by administering tritiated thymidine to the embryo [7]. The neuroepithelial cells that at the moment of injection are in a premitotic phase of the mitotic cycle thereby become heavily labeled. The daughter cells that originate from these cells receive, on average, half the label and they will pass it to the next generation in a similar way, thus diluting the label once again. If, however, soon after the first division following upon the administration of the label, the daughter cell appears to be a neuroblast (*i.e.*, a cell that is bound to leave the matrix and that is incapable of further division) this cell will retain its label and it will differentiate into a neuron. The nucleus of this neuron, then, is 'branded for life' (Angevine, '70). In this way it is possible to determine the day of birth (*i.e.*, of differentiation from a neuroepithelial cell into a neuroblast) of a neuron. Thus one can establish the day the neuron precursor looses its capacity to divide.

In this chapter an attempt is made to determine the sequence of origin of the neurons that make up the grisea of the diencephalon and to follow the differentiating neuroblasts on their migrations. The design of the series of experiments is summarized in table III (p. 10) which furnishes data on the age of the animals on the day of injection and on the survival periods after which the animals were sacrificed. The developmental stages represented by the days

7. Details regarding the technical aspects of the experiments are given in chapter III.

of injection of tritiated thymidine span almost the entire period of neuroblast production in the diencephalic part of the central nervous system. It ranges from embryonic day ten to embryonic day eighteen.

The use of autoradiography as a tool in neuroembryologic research described above rests on the generally accepted assumption that exogenous tritiated thymidine will be incorporated into the DNA of the dividing cell. The conditions that have to be fulfilled in order to get a proper incorporation have been discussed extensively in the literature (Rogers, '67; Cleaver, '67; Sidman, '70) and need not to be repeated here.

The criterion used in the ensuing description of the autoradiographs is the degree of labeling as expressed in the number of silver grains observed above the neuronal cell nucleus. In any single autoradiograph heavily labeled cells and a spectrum of moderately to lightly labeled cells are observed. The most heavily labeled cells we consider to have originated at the time of injection. All lightly labeled cells have to be disregarded as forming a mixed population. This population consists partly of younger cells which contain an amount of diluted label because of divisions subsequent to the injection, and partly of cells of the same age as the heavily labeled ones but that have been exposed to a lesser quantity of tritiated thymidine as a consequence of availability problems related to time and local distribution. Heavily labeled cells occasionally may appear as lightly labeled in the autoradiogram, due to technical difficulties in connection to section-emulsion problems. For an extensive discussion of these questions the reader is referred to Cleaver ('67), Rogers ('67) and Sidman ('70).

In the context of the experimental design of this study (with rather short intervals of times of injection of the different animals) the presence of both non-labeled and lightly labeled areas was of some help in tracing the time of origin of the populations of cells. Such differences in labeling offered the opportunity to situate these non-labeled and lightly labeled cells in relation to the heavily labeled ones as far as their time of origin is concerned.

In this chapter an answer must be found to the following questions, derived from the study of normal material:

— Is there a discrepancy, in general, between the conclusions drawn from the study of the normal material and the findings in autoradiographic series?

— In what order are radially migrating neuroblasts deposited in the mantle layer (inside-out or outside-in)?

— Is it possible to verify the direction of a 'tangential' migration (cf. p. 83) autoradiographically?

With regard to the development of certain particular diencephalic regions we tried to find an answer to a number of additional questions:

— Do the tegmentum and the prerubral tegmentum develop as one homogeneous structure, or are there regional differences in time of origin of the neurons?

– Which diencephalic cell masses appear as the earliest differentiating structures?
– Are there local differences demonstrable within the pars dorsalis thalami as far as the time of origin of the neurons is concerned?
– Do the autoradiographic experiments confirm the retardation of epithalamus, pars dorsalis thalami, regio postoptica and regio preoptica as observed in the matrix and mantle layer studies described in the preceding chapters?

OBSERVATIONS

In the presentation of the additional information obtained from the autoradiographic material the same sequence is maintained as in the description of the results of the study of normal development. This means that we will proceed from caudal to rostral.

After a careful scrutiny of the material collected at all stages of development with respect to the questions posed above, it appeared to be sufficient to confine ourselves to those groups of animals that, after having been injected in various successive stages of gestation, were allowed to survive to embryonic day 18 and postnatal day 100, respectively.

1 THE SYN-MESENCEPHALIC BOUNDARY ZONE AND THE SYNENCEPHALON

The syn-mesencephalic border as defined by Palmgren ('21) and described in chapter VII for the E_{18}-stage, forms a curved plane, rostrally convex in the dorsal region just caudal to the commissura posterior and rostrally concave in the basal parts where it is situated just rostral to the nucleus ruber and substantia nigra.

The boundary zone between tegmentum mesencephali and prerubral tegmentum. Basally in the E_{12-18} series [8]) labeled cells are observed near the midline located in the oculomotor and trochlear primordial nuclei and within the tegmental cell cord in more rostral regions (fig. 59). Only at the E_{13-18}-stage the nucleus ruber gets labeled. This structure becomes almost negative at E_{14-18}. During this and later phases of development no label can be observed in the mesencephalic tegmentum apart from a midventral cell-stream that extends from the fovea isthmi, located ventricularly at the mes-rhombencephalic border, to the top of the plica encephali ventralis externally. From there the cells spread in a rostral and lateral direction and become localized superficially in the basal syn-mesencephalic region. They constitute the nucleus interpeduncularis and also, more rostrally, the prerubral tegmental field of Tsai and part of the substantia nigra

8. The series of the autoradiographic experiments are characterized by the index E and two numbers; the first number indicates the time of administration of the tritiated thymidine; the second number indicates the day the animal was killed.

(fig. 60). The production of cells within this cell-stream can be observed to continue from E_{14} through E_{16}.

The prerubral tegmentum. Within the rostral continuation of the tegmentum mesencephali into the synencephalon no circumscript cell condensations were described other than the nuclei of Cajal and Darkschewitsch (labeled in E_{13-18}),

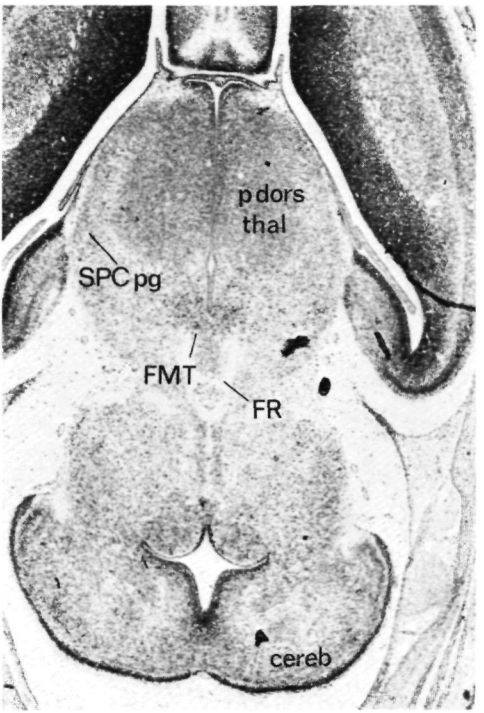
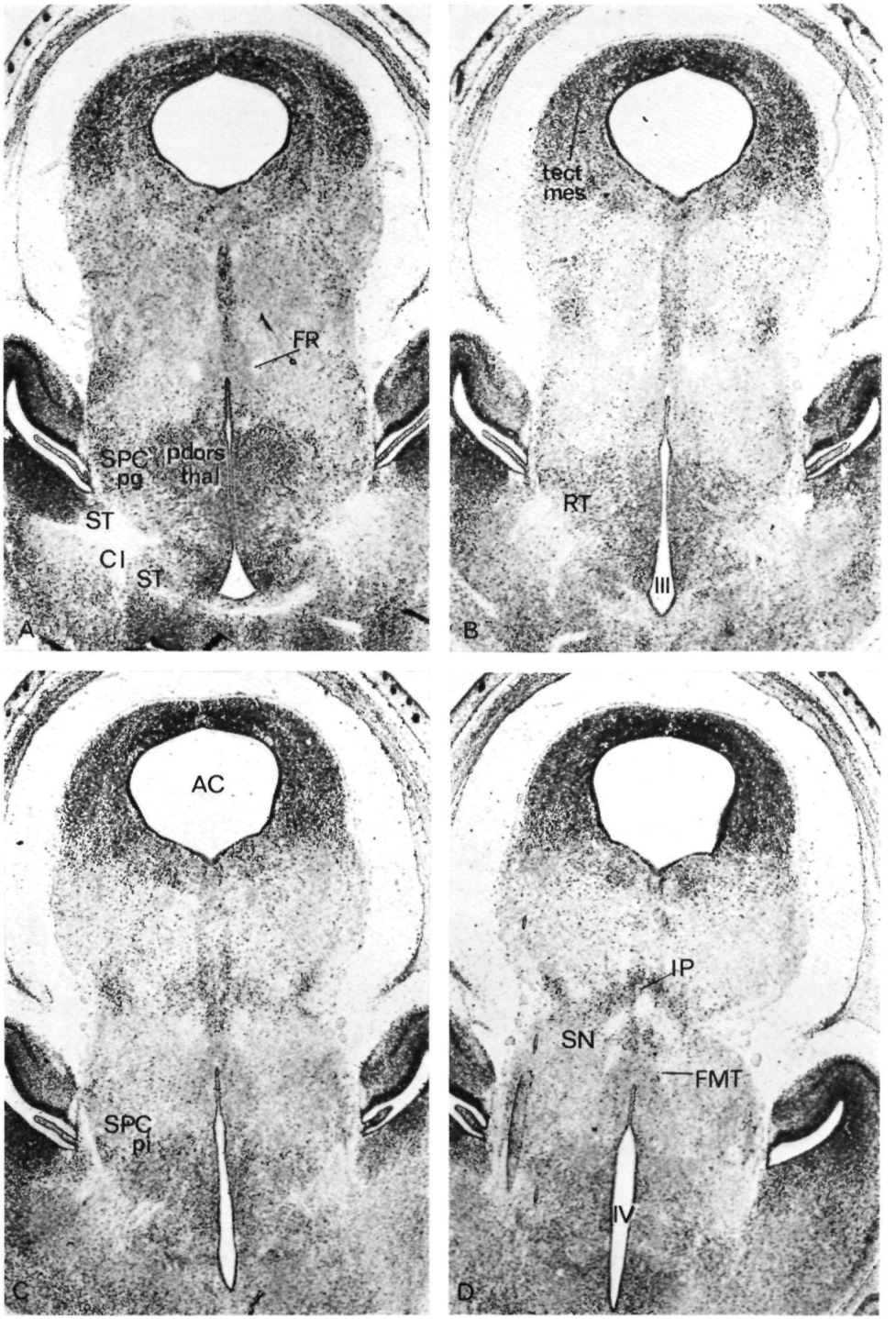


Fig. 59 Autoradiograph of a horizontal section through the syn-mesencephalic border region in an E_{12-18} series. The tegmental cell cord within the synencephalon shows heavily labeled cells.

which are considered to be derivatives of the tegmental cell cord, and the median nucleus of Perlia (nucleus medianus anterior) (labeled in E_{12-18}). In the basal regions the prerubral tegmentum attenuates between the caudal substantia nigra and the regio subthalamica in front of it; this thinner part of the prerubral tegmentum is also labeled in E_{13-18}.

Fig. 60 a b c d Autoradiographs of horizontal sections through the tegmentum mesencephali and the syn-mesencephalic boundary zone in an E_{14-18} specimen. Heavily labeled cells can be followed in the midline from a dorsal tegmental position (fig. A) to the top of the plica encephali ventralis (fig. D) where a spreading of cells in a lateral direction is observed.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The boundary zone between tectum mesencephali and pretectum. The site of earliest labeling of this dorsal region is situated within the realm of the caudal fibres of the posterior commissure at E_{12-18} and takes a rostrally convex form, thus justifying the supposition that its position is coincident with the early syn-mesencephalic interneuromeric crest. From here the label spreads in both rostral and caudal directions. As far as the mesencephalic tectum is concerned an early raphelike labeling in the midline is observed all along its course. Apart from this early developing structure two gradients were observed in the origin of the tectal neurons; first, a basodorsal one, corresponding to the tendency of histogenesis in the neural tube to accelerate basally and to retard dorsally. The second gradient concerns the retardation of the more caudal parts; thus the colliculus inferior becomes the last developing structure within the mesencephalon.

Pretectum. The dorsal part of the synencephalon, also called the pretectum, can be clearly divided into a commissural part and a precommissural part.

Considering the pretectum as a whole, both a basodorsal and a caudorostral gradient are observed in the pattern of labeling throughout the series.

Within the pars commissuralis a mediolateral gradient is observed in the nucleus centralis subcommissuralis and within the nucleus interstitialis magnocellularis, which are labeled at E_{13-18} and E_{14-18} respectively.

A mediolateral gradient is also observed in the pars precommissuralis at E_{14-18}.

At E_{15-18} and E_{16-18} the labeled cells of the pretectum are located exclusively in the rostrodorsal part of the pretectum (cf. fig. 61).

II PARENCEPHALON POSTERIUS

The subthalamic region. In the preceding chapter the regio subthalamica was defined as a basal constituent of the parencephalon posterius. Caudally it is continuous with the prerubral tegmentum and rostrally it extends into the basis of the parencephalon anterius (cf. p. 89). This subthalamic region is characterized by an early onset of the differentiation. The tegmental cell cord that extends rostrally beyond the fasciculus retroflexus is labeled at E_{12-18}, together with the most basocaudal part of the suprapeduncular complex. The rostrodorsal part of this region contains an early differentiating periventricular nucleus which we have called the nucleus hypothalamicus posterior. From these medially located structures the label spreads in a lateral direction. One day later in development the cells are labeled that are intermingled with the longitudinal fibre tracts passing through the regio subthalamica and which make up a substantial part of this region. The most laterally situated cells show the less intense labeling characteristic of cells that have been produced later (E_{13-18}). This observation led us to assume a mediolateral gradient in the production of cells within the regio subthalamica. As far as the constituents of the suprape-

duncular complex are concerned, no conclusive evidence could be obtained to support the supposition of a tangential migration in a rostrodorsal direction as suggested in the description of normal series. However, the results are not at variance with this hypothesis.

The corpus subthalamicum Luysi can be observed very early during development and occupies a very superficial position at E_{13-18}. This nucleus shows label in its rostrocaudal part which shifts gradually in a more basal position to become exclusively concentrated in the matrix of the recessus supramamillaris at E_{16-18}, thus providing a very clear example of a nucleus originating by tangential migration (cf. figs. 63 and 64).

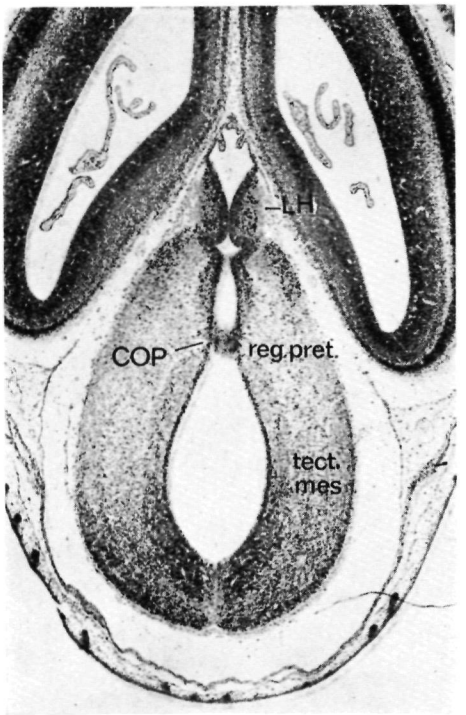

Fig. 61 Autoradiograph of a horizontal section through the dorsal part of the synencephalon in an E_{15-18} specimen. Within the pretectal region heavily labeled cells are present external to older, non-labeled cells.

Epithalamus. The epithalamic part of the parencephalon posterius shows a strictly caudorostral and basodorsal gradient in development. A rather uniform picture is observed in the E_{18}-stage all along the habenular region. The gradients just mentioned effect a difference in the position of the label that is slight as far

as the dorsobasal differences are concerned, but rather striking in the rostrocaudal direction. The order of appearance of the label is strictly lateromedial and the time of origin is protracted and ranges from E_{12-18} to $E_{18\ 18^1/_4}$ (cf. fig. 62a).

Pars dorsalis thalami. In the differentiation of this part of the parencephalon posterius, strictly caudorostral and basodorsal gradients prevail. The sequence of appearance of labeled cells is the outside-in variety. At E_{18} the pars dorsalis thalami still shows a homogeneous and basophilic aspect in most of its area. Only the somewhat more basocaudally located corpus geniculatum mediale and laterale show a more differentiated appearance. At E_{13-18} the first heavily labeled cells appear in the corpus geniculatum mediale and from this stage on the label gradually shifts forward and inward in a way that is governed by the three vectors just mentioned. E_{14-18} shows a labeled corpus geniculatum laterale and a partly clean corpus geniculatum mediale. At E_{15-18} the label has reached the rostral external surface. In E_{16-18} the superficial areas are rostrally unlabeled and in E_{17-18} a large part of the dorsal thalamic matrix is exhausted with the exception of the most rostrodorsal part adjacent to the velum transversum.

III PARENCEPHALON ANTERIUS

Pars ventralis thalami. Rostrally and dorsally this part of the diencephalon is characterized by the presence of a complex mass of fibre tracts. Caudobasally the pars ventralis thalami borders on the regio subthalamica; a rather early labeled nucleus hypothalamicus posterior (E_{13-18}) is located in the boundary zone.

The nucleus reticularis thalami becomes labeled in a basolateral to mediodorsal fashion. This nucleus gets squeezed between the pars dorsalis thalami and the diencephalic constituents, which are located more basally; therefore, a caudorostral gradient is hardly discernable (E_{14-18}). At $E_{14^1/_2-18}$ the basolateral part of the reticular nucleus is label-negative already and at E_{15-18} all parts of this nucleus are label-negative. The suprahypothalamic complex, *i.e.*, the remaining part of the pars ventralis thalami (which is located in a juxtaventricular position) is labeled in the E_{15-18} specimens and gives origin to the rostral part of the zona incerta.

IV THE CONSTITUENTS OF THE HYPOTHALAMUS

Corpus Mamillare. The cells produced in the mamillary region give origin to both the mamillary nuclear complex and the premamillary nuclei. The mamillary nuclei originate from a matrix adjacent to the ventricular protrusion just rostral to the recessus supramamillaris. From the matrix of the recessus supramamillaris itself the corpus subthalamicum Luysi develops. The mamillotegmental tract which can be recognized already at an early stage indicates the

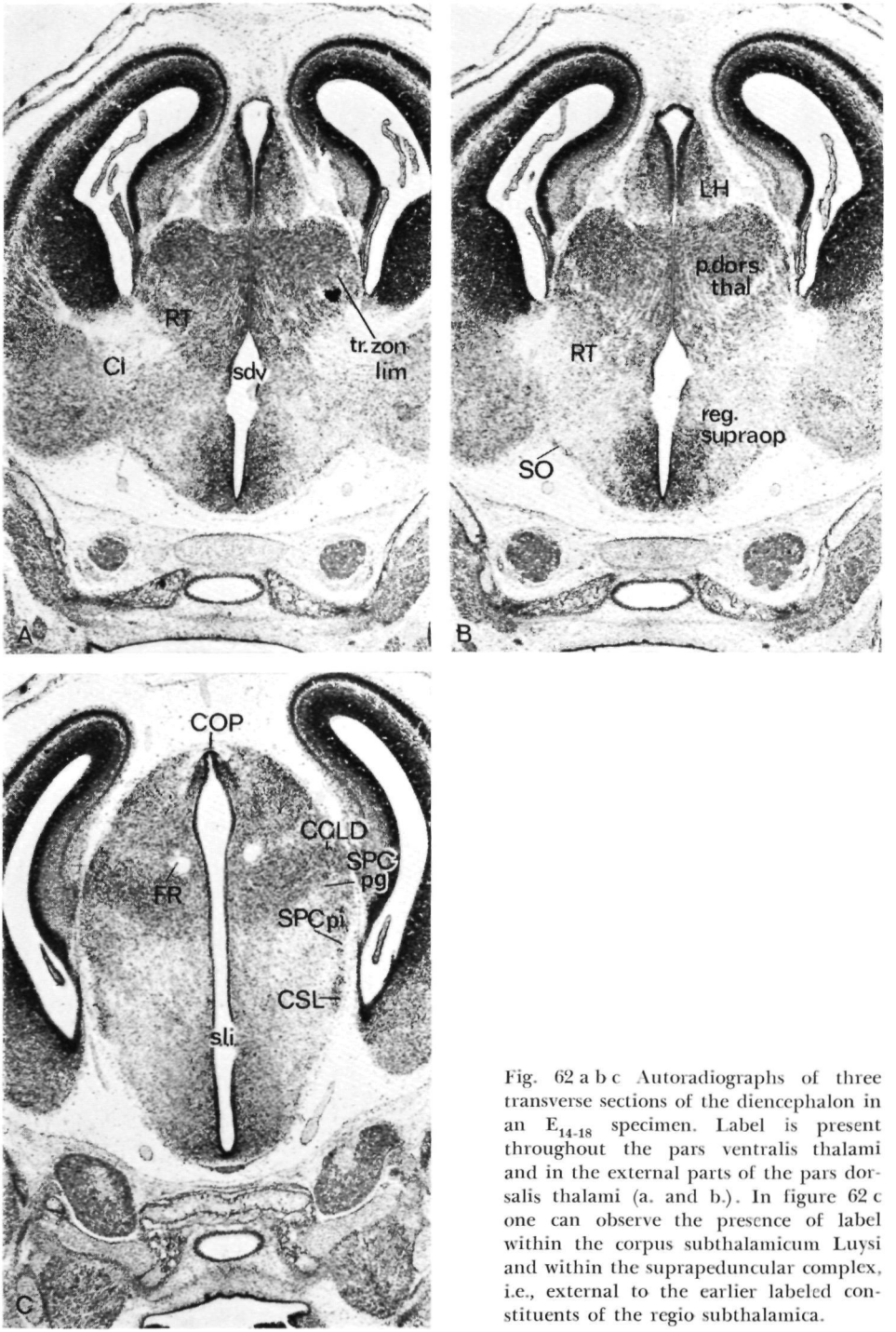

Fig. 62 a b c Autoradiographs of three transverse sections of the diencephalon in an $E_{14\text{-}18}$ specimen. Label is present throughout the pars ventralis thalami and in the external parts of the pars dorsalis thalami (a. and b.). In figure 62 c one can observe the presence of label within the corpus subthalamicum Luysi and within the suprapeduncular complex, i.e., external to the earlier labeled constituents of the regio subthalamica.

position of the mamillary anlage. Slightly rostral to this internal mamillary tubercle, the matrix located in the midline produces cells that migrate tangentially towards a superficial position. The cellular condensations thus formed are the primordial premamillary nuclei.

As far as the sequence of development of the mamillary nuclei is concerned, we observed that the lateral mamillary nuclei are produced at the E_{13}-stage. In E_{14-18} the label approaches the lateral surface of the mamillotegmental tract and at E_{15-18} it is located exclusively medial to this tract. Thus a strictly lateromedial gradient can be observed within the mamillary complex. The premamillary nuclei are seen to develop prior to the mamillary complex. It is noteworthy that the anlagen of the mamillary and the premamillary nuclei in the autoradiographic series exhibit a characteristic pattern, consisting of alternating 'advanced' and 'retarded' zones (fig. 64).

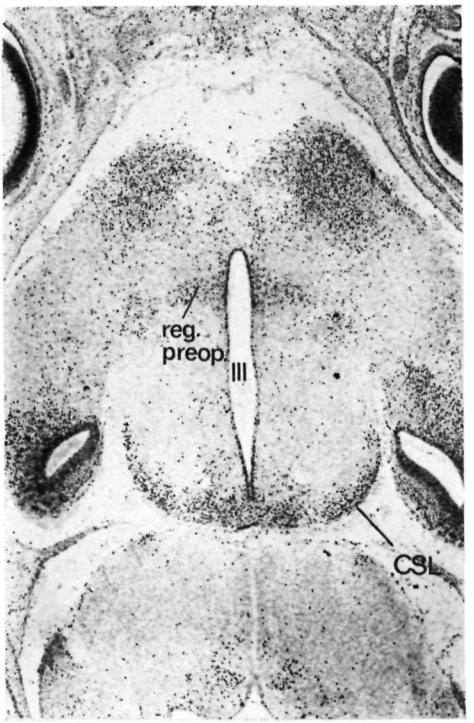

Fig. 63 Autoradiograph of a horizontal section through the subthalamic and hypothalamic regions. The corpus subthalamicum Luysi can be clearly observed in a superficial position and heavily labeled cells can be discerned in its rostrodorsal part (E_{15-18}).

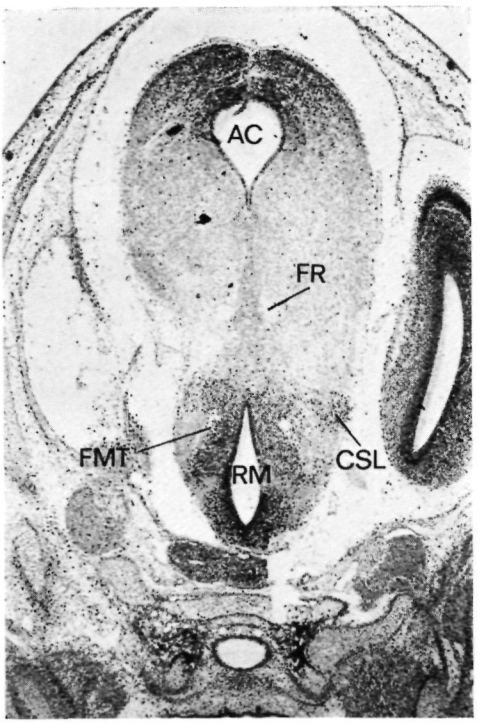

Fig. 64 Autoradiograph of a transverse section passing through the mesencephalon and through the caudalmost part of the third ventricle. Note the peculiar picture presented by alternating heavily labeled and mature, unlabeled areas in the hypothalamic part of the figure. (E_{15-18}).

Regio postoptica. The cells that are labeled early are located in rostral and lateral positions (E_{12}, E_{13}). Within this region therefore a lateromedial labeling pattern is observed. This pattern is not so evident in the rostral border of this region. Here an early differentiated boundary zone with the regio supraoptica exists (E_{12}, E_{13}); moreover the nucleus ventromedialis hypothalami shows medial labeling, while at the same stage of development elsewhere the label reaches the lateral surface. This applies to both the E_{14-18} and E_{15-18}-stages.

At the E_{16-18}-stage the labeling is confined to the matrix adjacent to the sulcus lateralis infundibuli. At E_{17-18} and $E_{18\ 18}1/_1$ the label is only present in a strictly periventricular position.

Regio supraoptica. A diffuse suprachiasmatic nucleus that crosses the midline in the boundary-zone between regio postoptica and regio supraoptica is labeled at the E_{12}-stage. In front of this boundary-zone of the supraoptic region with the postoptic region, no label can be observed at E_{12-18} except in the most lateral parts where the interstitial nucleus of the medial forebrain bundle is present. In the E_{13-18} series both the nucleus paraventricularis and the nucleus supraopticus are labeled. There appears to be a strand of cells between the two primordia which separates the lateral parts of the hypothalamus (*i.e.*, the interstitial nucleus of the medial forebrain bundle) from the medial parts. The area medial to this early differentiating zone is interpreted as the nucleus hypothalamicus anterior (lateral and dorsal) and the nucleus supra-chiasmaticus (medial and basal). These nuclear groups are labeled in the E_{14-18} series. The E_{16}-stage is clearly a final stage and only ventricularly shows some scattered labeled nuclei. The overall impression is that the supraoptic region develops later than the postoptic region.

Regio preoptica. Rostral to the supraoptic region and rostral to the sulcus intraencephalicus anterior, the preoptic region is a diencephalic area bordering on the telencephalon. Its lateral, early labeled parts (E_{12-18}) touch on the diagonal band of Broca and they are caudally continuous with the interstitial cells of the medial forebrain bundle. From there on the label gradually becomes located closer to the ventricular surface. Thus it is visible in the lateral part of the nucleus preopticus medialis in E_{13-18} and juxtaventricularly at E_{15-18}. Later on in the matrix a labeled cell can be observed only occasionally (E_{16-18}, E_{17-18}).

In general it can be said that in the preoptic region a dorsobasal and a caudorostral gradient are observed and that the depositing of the nuclei occurs in a lateromedial sequence.

Thus the dorsorostral border of the preoptic region, which coincides with the torus hemisphaericus, is progressive and its matrix is exhausted earlier than in the remainder of the preoptic region.

For a long time, the matrix surrounding the preoptic recess remains strongly basophilic, but autoradiographically proven cell multiplication appears to be very rare indeed in the period beyond E_{16}.

Thus the results of the autoradiographic part of this study confirm the existence within the hypothalamus of four zones. Fig. 65, a horizontal section through the hypothalamus, shows the four regions in their rostrocaudal sequence which results from the morphogenetic transformations of earlier stages.

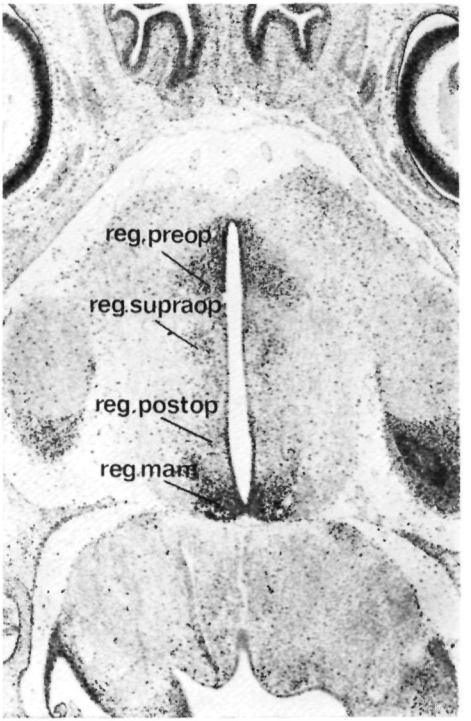

Fig. 65 Autoradiograph of a horizontal section through the hypothalamus. Labeling can be observed in the centre of each of the four ontogenetic units described in the text (E_{15-18}).

V THE TELODIENCEPHALIC BORDER-ZONE

A number of grisea is present in the caudobasal half of the telodiencephalic border-line. Dorsorostrally this border is characterized by membranous structures, as was described earlier. The median torus transversus is marked by the presence of a commissura anterior and its bed nuclei (E_{13-18}).

The anterolateral part of the hypothalamus (represented by the nucleus preopticus pars lateralis) and the nucleus of the diagonal band of Broca are labeled in the E_{12-18} series; lateral to this area a field of negative and weakly labeled cells is observed that later on appears to develop into the tuberculum olfactorium and substantia innominata. These structures only become heavily

labeled at E_{14}. A histogenetic relationship was observed between the cells of the olfactory tubercle and the matrix of the olfactory ventricle. The laterally located structures discussed thus far occupy a relatively superficial position. Dorsal to them and close to the ventricle the globus pallidus and the nucleus centralis amygdalae are found. These structures appear to have ontogenetic connections with the diencephalon, as far as the time of origin of their neurons is concerned (E_{12-18}, E_{13-18}).

DISCUSSION

In the autoradiographic part of this study, the time of final division of the neurons within the diencephalon was studied. A detailed discussion of the time of neuron origin in every single nucleus of the diencephalic subdivisions is outside the scope of this report. Such studies have been made by Angevine ('70) for the dorsal diencephalic structures in the mouse and by Ifft ('71) for the hypothalamic nuclei in the rat. They provide important information that has to be evaluated together with the data obtained from morphogenetic and hodogenetic studies.

The additional information obtained from the autoradiographic part of this study mainly involves internuclear differences in time of neuron origin and the gradients of differentiation that can be deduced from these differences and that are characteristic for any particular subdivision of the diencephalon.

The early differentiation within the tegmentum mesencephali, the prerubral tegmentum and the subthalamic region as observed in the study of the mantle layer, is fully confirmed.

Within the tegmentum mesencephali, a median stream of labeled cells was noticed which originates from the region of the fovea isthmi and pierces the tegmentum in order to contribute to the formation of the nucleus interpeduncularis, the ventral tegmental area of Tsai and the substantia nigra. This phenomenon has been described by Cooper ('46) in a human embryo and was confirmed autoradiographically by Hannaway *et al.* ('71), whose publication appeared during the preparation of this manuscript. The stream of cells behaves as a 'fountainhead' that perforates the early formed tegmentum to deliver cells which become located in a basal position. A certain proportion of the cells maintains a basal, superficial position, but others continue to migrate and shift lateralward. The cells that remain in the basal position constitute the nucleus interpeduncularis and the prerubral field of Tsai; the cells which migrate to a lateral position contribute to the formation of the substantia nigra. This mode of formation of nucleus interpeduncularis, prerubral field of Tsai and substantia nigra complicates the structure of the basal synencephalic area considerably. In this area the tegmental cell cord derivatives should be considered the only structures of a genuine synencephalic origin. The dorsal part of the synencephalon exhibits

a general caudorostral and mediolateral (inside-out) gradient of labeling.

Rostral to the par-synencephalic border a marked difference of development is observed between the basal parts, *i.e.*, the subthalamic region and the pars dorsalis thalami and epithalamus. The regio subthalamica is characterized by a mediolateral (inside-out) gradient of labeling. There are some indications that the mode of migration in which the more laterally placed structures in the subthalamic region develop, closely resembles that which was observed in the tegmentum mesencephali and which was indicated above as the midbasal 'fountainhead'. In the case of the corpus subthalamicum Luysi this could be clearly observed. Within this nucleus a dorsobasal gradient was demonstrated.

Both in the pars dorsalis thalami and in the epithalamus the dorsal part of the parencephalon posterius shows a caudorostral and a basodorsal gradient, and the labeled cells appear in a lateromedial sequence. The pars dorsalis thalami is considerably retarded when compared to the surrounding structures. Until the E_{18}-stage this region develops in a uniform way without a clear distinction between nuclei or pronuclei being possible. Both the mantle layer study and the autoradiographic data suggest that the corpus geniculatum mediale belongs to this thalamic mass. Its caudobasal position can probably be attributed to the early differentiation of this part of the pars dorsalis thalami that thereby becomes "fixed" to the tegmental structures of synencephalon and parencephalon posterius, which also develop relatively early.

Our autoradiographic data fully confirm Angevine's ('70) conclusions as far as the internuclear differences in time of neuron origin are concerned. We observed the existence of a basodorsal and a caudorostral gradient in the lateromedial mode of appearance of neurons within the pars dorsalis thalami (we remind the reader that the use of the terms of orientation in this study is always relative to the morphological position of the structure at issue) and the same is true for the epithalamus. Angevine also suggested the revectoring of an early rostrocaudal gradient of development into a 'ventrodorsal' one which, as the author supposed, should be brought about by a rearrangement of neuromeric components of the diencephalon into Herrick's four longitudinal zones. No such revectoring could be observed, however, in the extensive material at our disposal. Neither could we explain the caudorostral gradient as a redirected 'ventrodorsal' gradient as found in earlier stages as was suggested by Angevine. According to our observations the differentiation process is seen spreading from the caudobasalmost points of both epithalamus and pars dorsalis thalami in a dorsal and a rostral direction, and this occurs at a developmental stage at which the neuromeric borders can still be clearly recognized.

The same gradients were observed within the pars ventralis thalami which is located dorsal to a continuation of the regio subthalamica. The nucleus reticularis thalami, therefore, shows a strong basodorsal and a lateromedial gradient.

As far as the distribution of the label in the hypothalamus is concerned, its

heterogeneous embryonic origin as described in earlier chapters, is clearly reflected in the autoradiographic picture.

The premamillary nuclei develop prior to the corpus mamillare itself in a position adjacent to the regio postoptica. Within the corpus mamillare the localization of the label follows a lateromedial gradient from E_{13} through E_{15}.

The regio postoptica is bordered by early differentiating zones, the premamillary nuclei caudally and the nucleus suprachiasmaticus diffusus and the interstitial nuclei of the supraoptic commissures rostrally. Within this region the lateral cells are labeled at early stages. The nucleus ventromedialis is an exception to the general lateromedial gradient within this region, as it already shows strongly labeled cells in its medial half at E_{14}.

The supraoptic region is characterized by the early differentiation of the nucleus paraventricularis and nucleus supraopticus. In a number of series these two nuclei can be observed to be connected by a strand of cells; both the cells located laterodorsally and those located mediobasally to this structure differentiate later on.

Within the preoptic region a strictly lateromedial gradient exists, the label appearing first in the dorsocaudal region. Proliferation continues rather late, as can be concluded from the fact that at E_{17-18} scattered label still can be observed in the matrix rests.

Our results do not contradict the data obtained by Ifft ('71) in the rat as far as the time of origin of separate nuclei is concerned. He, however, also observed a general lateromedial and a possible 'dorsoventral' gradient within the hypothalamus as a whole. In our opinion, however, it is not feasible to try to describe gradients for the hypothalamus as a whole. The present study has clearly shown that the hypothalamus cannot be regarded as a fundamental unit. Hence a valid description of gradients should be restricted to each one of the four ontogenetic units that together constitute the hypothalamus.

The telodiencephalic border-zone is characterized by the presence of the nucleus diagonalis of Broca which was found labeled in an early stage. Both the globus pallidus and nucleus centralis amygdalae are also early differentiating structures of this level of the brain.

We are now in a position to answer the general questions posed in the introductory paragraph of this chapter. The early appearance of a mantle layer configuration in the normal material corresponds very closely to the early appearance of labeling in the autoradiographic series in the particular region under consideration. The longstanding preservation of a basophilic appearance, which is a characteristic feature of the matrix areas of the developing neural tube in normal material, on the other hand, does not necessarily implicate the presence of an actively proliferating matrix cell population, as is exemplified by the preoptic region.

The appearance of labeled cells in the mantle layer derivatives of a particular region in general occurs in an 'outside-in' sequence. Within the regio pretectalis and the regio subthalamica, however, an 'inside-out' sequence was observed. The mechanism of this 'inside-out' sequence is not wholly understood. Observations in series which were sectioned in a favourable direction suggested the presence of both 'progressive' and 'retarded' areas in these regions. A curved plane of migration in the 'retarded' areas might furnish an explanation for the observations in later stages of label external to the areas which were differentiated earlier.

A strictly 'tangential' migration as was observed in the case of the origin of the corpus subthalamicum Luysi can be considered an extreme example of such a migration in a curved plane, which occurs just underneath the external surface. Those cells of such a 'tangential' migration that are labeled early, are observed in a position farther away from their matrix of origin than later labeled neuroblasts within the same cell stream. It thus can be concluded that, if the way of migration is taken into consideration, all mantle layer structures within the diencephalon do originate in an essentially 'outside-in' fashion.

The way in which the median 'cell stream' observed in the tegmentum mesencephali gives origin to the nucleus interpeduncularis, the prerubral field of Tsai and part of the substantia nigra, shows a resemblance to the development of the corpus subthalamicum Luysi and to the way in which the premamillary nuclei develop. It therefore seems justified to pose the question whether the upside-down 'fountainhead' migration observed in these locations is a characteristic of the unpaired primary continuous part of the neural plate that reaches from the fovea isthmi to the chiasma opticum.

In general it can be concluded that the study of tritiated thymidine autoradiography has added the dimension time to the otherwise static histological picture presented by the sectioned series. On the other hand, the conclusions reached by Angevine ('70) and Ifft ('71), in our opinion, provide evidence that the interpretation of autoradiographs of the adult brain without study of the early embryonic stages is dangerous. It was the combined study of both normal and autoradiographic, embryonic and adult specimens that made autoradiography a valuable tool in our study of diencephalic development.

CHAPTER IX

DISCUSSION

INTRODUCTION

In this chapter an attempt is made to present a synthetic review of the results obtained in the present study. The investigation of the ontogenesis of the diencephalon in the Chinese hamster was undertaken in order to test in one single species the validity of the various criteria of subdivision of the brain as employed by different authors. Therefore the morphogenetic and histogenetic phenomena and their interrelations are discussed and our results are compared with the data mentioned in the literature. (For a specification of the phenomena that were the object of study, the reader is referred to table I).

From this discussion a general picture of the structural plan of the diencephalon of the Chinese hamster results and an opinion is advanced as to the consistency of the subdivisions obtained from the application of the different criteria. A discussion of the concept of homology was considered beyond the scope of the present study and has intentionally been left out of consideration.

The discussion is complemented by a regional analysis of the diencephalon of the adult Chinese hamster in the light of the results obtained in the previous chapters of this study.

TRANSFORMATIONS IN THE MEDIAN PLANE; TERMINOLOGY OF ORIENTATION

A pilot study of sagittally sectioned series revealed important morphological transformations of the neural tube during development (see fig. 4). The neural tube as a whole shows a number of flexures (flexura cephalica, flexura cervicalis, flexura pontis). This makes the analysis of the brain from a study of series sectioned in the conventional planes extremely difficult, as the morphologically transverse plane will be different for each part of the neural tube. Accordingly we became convinced that a reliable study of the part of the brain under consideration could not be performed without a wide application of reconstruction techniques.

In order to have a clear terminology at our disposal, we strictly held to the convention according to which the neural tube is thought of as unrolled. The terms 'rostral' and 'caudal' were used for differences in localization along the

central brain axis. The terms 'basal' and 'dorsal' were also used as referring to this central brain axis irrespective of the position of the structure under consideration within the embryo as a whole.

The study of the midline configuration revealed that the structures developing here retain a mutually constant position while undergoing topological transformations. They therefore are considered landmarks and together they form a kind of reference scheme that can be used in comparative studies.

The transformations of the structures in the wall of the third ventricle that were described show a rotation movement not unlike the movement of a transport band at its turning point. This movement causes a caudodorsal shift of the point where the central brain axis ends in the terminal wall of the third ventricle. As a consequence, the angle between the main longitudinal axis of the rhombencephalon (the axis of Meynert) and the main longitudinal axis of the prosencephalon (the axis of Forel) shows a gradual increase during development, which results in the 'stretching' of the brain as was also reported in other species by Haller ('29), Spatz ('35), Luyendijk ('44) and Grunthal ('52).

After these remarks on the morphological and terminological orientation we shall first define the boundaries of the part of the brain which forms the object of our study. We shall then present a discussion of the problems related to the description of boundaries in the nervous system in general and attention will be paid also to the mesodiencephalic and to the telodiencephalic border-zones.

THE MESODIENCEPHALIC AND THE TELODIENCEPHALIC BORDER-ZONES

When considering boundaries within the brain, almost always reference is made to the relatively simple embryonic condition. Investigators tend to resort to the neuromeres or to the neuromere-like 'five-vesicle stage' and to subdivide the adult brain according to the 'parts' obtained in this way. When first presented (His, 1888) this subdivision of the brain meant an advance on anything that was published before, not only because it offered a reference scheme to be used in comparative neuroanatomical investigations, but also because the model of the nervous system thus created could be used for didactic purposes. However, since posing the problem in the field of comparative neuroanatomy was more and more focussed on questions of homology, it appeared that those boundaries which in the early ontogenetic stages could be readily established, became problematic during later phases of development. For, where in early stages the ventricular and pial surface of the cerebrum have a simple relationship, during later stages of development these surfaces move apart to such a degree that it is not clear any longer whether grisea in the mantle layer belong to one or the other of two neighbouring divisions of the cerebrum. There are several ways out of this problem. For instance it is possible in such a situation to speak of a

transition area or border-zone. Another possibility is, if both an internal ventricular and an external pial border-line can be discerned, to connect these lines and to denominate the plane thus formed the border-plane between the adjacent parts of the brain. If, however, both border-lines are not parallel, the position of this border-plane is not clear. It is still less exact to indicate intracerebral boundaries by just offering a dorsal and a basal midsagittal landmark and stating that the transversal plane through both points is the boundary-plane. The two latter ways of boundary-indication do not take into account the transformations to which the boundary-planes are subject in the course of development and which are not limited to one out of three dimensions.

In our opinion, the only correct way to acquire knowledge of the boundary-planes, is to trace the grisea from their earliest time of origin in the wall of the brain tube. In this way the natural boundary-planes, if present, can be detected, and these natural boundary-planes should function as landmarks within the field of comparative neuroanatomy.

This opinion was formulated in 1921 by Palmgren: "It is to be expected that during the process of development a boundary-plane, which from the beginning is level, becomes more or less curved, as some parts develop more than others. It is thus clearly impossible in such a case, that boundary-grooves and corresponding interneuromeric ridges should alone suffice. Straight lines drawn up between these (on sections) are boundary-lines only in case of the boundary-surface being level. If this surface later becomes curved, these lines become entirely artificial, and only approximate as boundary-lines". After some remarks on histogenetic features Palmgren continues: "A natural boundary should therefore run parallel to the rows of cells, ependymal fibres and in certain cases bloodvessels" (p. 24). The number of authors who explicitly subscribe to this point of view is rather limited (Tandler and Kantor, '07; Sterzi, '12; Schumacher, '28). We are able to confirm the importance of the concept expressed in the remarks cited above from the vantage point of the study of the Chinese hamster brain.

The caudal boundary of the diencephalon

Opinions concerning the border-zone between mesencephalon and the caudal part of the diencephalon (synencephalon) show some variation. A number of causes can be inculpated that have made the study of this region rather difficult.

In early phases of development the cranial flexure is located at the mesodiencephalic transition; consequently the natural transverse planes of this part of the neural tube undergo considerable changes in position over a short distance (Kuypers, '52). In addition, changes occur in the sagittal plane that can be formulated succinctly: the dorsal part of the mesencephalon seems to be in the process of moving across its base in a caudal direction; the tegmentum mesencephali

is translocated rostrally and becomes situated, partly at least, in the rostral limb of the plica encephali ventralis (Tandler and Kantor, '07).

Authors at the beginning of the century (His, 1893, '04; Von Kupffer, '06) contented themselves with the indication of a dorsal and a basal median point, thus fixing the transversal border-plane between mesencephalon and diencephalon. Later investigators demanded that the transformations in the lateral parts of the neural tube should also be taken into consideration (Tandler and Kantor, '07; Palmgren, '21; Rendahl, '24). Both morphogenesis and histogenesis of this part of the brain were studied in relation to this question. As far as the midline points are concerned, the dorsal part of the boundary was fixed in front of (Orr, 1887; Haller, '29; Herrick, '48; Kuhlenbeck, '54) or behind (Neal, 1898; Von Kupffer, '06; Palmgren, '21; Vaage, '69) the posterior commissure. Basally the border was considered to pass through the tuberculum posterius (His, 1893) or behind this structure (Von Kupffer, '06) or, as was the opinion advanced by Neal (1898) and Palmgren ('21), just in front of the fibres of the oculomotor nucleus.

The morphogenetic approach of the caudal diencephalic boundary rests upon the appearance of constrictions in the wall of the neural tube, *i.e.*, of interneuromeric borders. The eventual di-mesencephalic boundary corresponds to the early fissura syn-mesencephalica that develops in the neuromere stage (for a discussion see Palmgren, '21; Vaage, '69). In the basal parts of the brain this constriction disappears during development. The enormous thickening of the tegmental parts renders these external characteristics marginal phenomena. Therefore, such a superficial delimitation should be supplemented by a description of the histological appearance of this syn-mesencephalic border in the wall of the brain tube.

Palmgren ('21) analysed the development of the posterior commissure which, according to him, is located just in front of the syn-mesencephalic constriction. The secondary ventricular prominentia di-mesencephalica which is raised by the posterior commissure should therefore be considered entirely diencephalic. The tuberculum posterius region lies, according to Palmgren, far in front of the syn-mesencephalic border and a line from the commissura posterior to the tuberculum posterius cuts obliquely through the synencephalon. He maintains a basal syn-mesencephalic border which passes just in front of nucleus oculomotorius, nucleus ruber and substantia nigra. We found no arguments to refute this opinion. In tracing this boundary-plane, however, we met with considerable difficulties, caused by the well-known crowding of cells of the mammalian embryonic brain. If the nucleus ruber and the substantia nigra are defined as mesencephalic structures the basal syn-mesencephalic boundary-plane can be identified by 'retrograde' studies. In older embryonic stages (*e.g.*, E_{18}) the shape of the syn-mesencephalic boundary-plane is a very peculiar one. In the midline the dorsal and the basal half meet at a rostrally obtuse angle. If the boundary-plane is followed in a lateral direction, it is seen that the dorsal part slopes in a caudal direction

while the basal part slopes in a rostral direction. Thus the whole of this boundary-plane is curved with the concavity directed caudally in its dorsal half and the concavity directed rostrally in its basal half. In figure 58 the syn-mesencephalic boundary-plane is illustrated diagrammatically.

The verification of these observations is best made in so-called horizontally sectioned series. A comparable observation was made by Rendahl ('24) in chick embryos.

The rostral boundary of the diencephalon

If an historical approach is followed in the discussion of the rostral diencephalic border, it can be observed that its definition has changed considerably in the course of time. In order to be able to visualize the configurations that will be described the reader is referred to figures 17-25 which represent reconstructions of the ventricular surface in the E_{12}, E_{13} and E_{14} Chinese hamster embryo.

According to His (1888) the di-telencephalic boundary is marked by an external fissure which divides the original prosencephalon into two parts. This constriction of the neural tube begins dorsally at the level of the velum transversum, curves around the neural tube and crosses the basal midline structures between the infundibulum and the mamillary anlage. This division implies the inclusion of the pars optica hypothalami in the telencephalon. This opinion was shared by Neumayer (1899), Weber ('00), Kamon ('06) and Ziehen ('06).

Johnston ('09), however, argued that the recessus postopticus was erroneously identified by His with the infundibular anlage. Thus the basal representation of the di-telencephalic border of His should cross the midline in between the postoptic recess and the infundibular anlage. The infundibulum was restored to the diencephalic part of the prosencephalon. His's misinterpretation of this early intraprosencephalic border is quite understandable; at the stage at which the border in question is most clearly visible any histological differentiation in the wall of the neural tube fails; it is therefore very difficult to trace the ventricular crest that is formed by this interneuromeric constriction in relation to the persisting diencephalic structures in later stages. In our material we succeeded in obtaining evidence sustaining Johnston's view (cf. figs. 17, 18 and 19). In the discussion on neuromerism we will consider this subject in some detail.

Vaage ('69) failed to grasp the importance of Johnston's correction of the original telodiencephalic border of His and describes its basal part in the same location as did His.

An internal boundary sulcus between telencephalon and diencephalon was described by Von Kupffer ('06), *i.e.*, the sulcus intraencephalicus anterior. The dorsal part of this telodiencephalic boundary is formed by the caudal margin of the foramen of Monro (cf. figs. 23 and 25). This border, which passes from

the velum transversum towards either the recessus preopticus or the anterior margin of the optic chiasm, is used in a number of present-day textbooks. (Peele, '61; Crosby, Humphrey and Lauer, '62; Strong and Elwyn, '64; and Ranson and Clark, '66).

A third opinion on the telodiencephalic boundary was presented by Mihalkovicz (1877), Meck ('07, '09, '10), Tandler and Kantor ('07) and Kuhlenbeck ('54, '56). They observed an external boundary sulcus which crosses the median plane dorsally through the velum transversum all the same. From there it takes a more rostral course, crossing the basal structures in front of the preoptic recess. A corresponding ventricular eminence can be followed that in early stages is called torus haemisphaericus. Later on a part of this eminence develops into the basal torus transversus, in which the anterior commissure crosses the median plane (cf. figs. 17-22).

All three telodiencephalic borders discussed above correspond as far as the presence of a telencephalon impar is concerned. These borders can be found, during some stages of development at least (Vaage, '69; this study) in the embryonic brain and it thus seems a matter of definition which of these boundaries should be preferred. In studying the literature on the development of the prosencephalon it thus appears to be of paramount importance to find out how the di-telencephalic boundary-plane is defined by the various authors.

If one tries to make a subdivision of the brain on the basis of neuromerism, the sulcus intraencephalicus anterior border has to be discarded, as this very remarkable structure does not coincide with an interneuromeric border. The other two boundaries, on the contrary, do correspond with such a neuromeric border. Unfortunately, 'the most natural border' (Vaage, '69) of His as corrected by Johnston was never wholly accepted. Probably this is due to the powerful impact of Kupffers ideas on his contemporaries. Another factor may be the position of the optic tract and the optic chiasm that traditionally have been considered to be diencephalic and intimately connected with the 'thalamus opticus'.

In this study the velum transversum-torus transversus boundary was traced from early embryonic stages to the adult brain (see chapters IV, V). It is this boundary that is meant when the telodiencephalic border is discussed below.

We will now turn to a consideration of the telodiencephalic border as it emerged from the morphogenetic and histogenetic parts of this study.

At the earliest stages of development, when a single bilaterally symmetrical telencephalic evagination originates, the border can be readily described; in the adult, however, a practically inextricable situation is observed. The torus haemisphaericus, running from velum transversum to torus transversus and manifesting itself externally as sulcus haemisphaericus (fig. 19), in the younger stages forms the caudal border of the foramen Monroi (figs. 17, 18). At this point we will first attempt to describe the development of the dorsocaudal part

of the border; the description of the rostrobasal half will be presented next.

The vicissitudes of the dorsal half of the telodiencephalic border are closely related to the origin of the plexus chorioideus of the lateral ventricle. The plexus develops in continuity with the anterior limb of the velum transversum. The posterior limb is also of an epithelial structure and is continuous with a lamina epithelialis that, morphologically speaking, is situated caudal to the plexus chorioideus. Because of the transformations of the prosencephalon this lamina epithelialis becomes involved in the formation of the medial wall of the hemispheric vesicle. In later stages it becomes the lamina affixa. If the development of this epithelial layer (the lamina affixa) is taken into consideration there is sound reason to call it a diencephalic structure. The lamina affixa extends to the caudal part of the lateral ventricle. Thus the diencephalon ectropionates into the hemispheric vesicle and the caudodorsal margin of the foramen of Monro of later stages is formed by morphologically diencephalic structures (*i.e.*, the eminentia thalami).

The rostrobasal half of the telodiencephalic border follows a quite different course of development. Here the telodiencephalic border-zone thickens considerably because of the expansive growth of a number of grisea and because of the passage of fibre bundles that gradually attain a relatively enormous size (Goldstein, '05). This mighty connection between telencephalon and diencephalon is often designated as hemisphere-stalk (Haemispherenstiel, Hochstetter, '19). Within this massive structure the telodiencephalic boundary cannot be indicated without difficulty. Ventricularly the sulcus terminalis is its representative. Within the hemisphere stem the globus pallidus, according to Spatz ('27) and Richter ('65) can be reckoned among the diencephalic structures. Thus the telodiencephalic border would run between pallidum and putamen, with a laterally directed convex surface. Rose ('42) even suggests the basal extension of this boundary just medial to the prepyriform cortex. Rostrobasally the border runs between the regio preoptica and the medial ganglionic eminence until it reaches the torus transversus, in which the commissura anterior develops.

In the histogenetic part of this study a confirmation of this telodiencephalic border was obtained in both the normal and in the autoradiographic part of the study. The floor of the ventricular sulcus terminalis is formed by a cellular structure, the massa cellularis reuniens. This massa cellularis reuniens can be subdivided into a pars superior and a pars inferior, which are continuous with the pars ventralis thalami and the regio preoptica hypothalami respectively. Within the amygdaloid complex the nucleus centralis has a number of features in common with the globus pallidus. Among these are the time of origin as demonstrated by the autoradiographic technique and also the type of cells.

The data from literature concerning the provenance of the globus pallidus show agreement about its diencephalic origin (Kuhlenbeck, '24; Spatz, '27, '49; Kahle, '56; Richter, '65). No evidence could be obtained that the matrix of the

medial ganglionic eminence provides its constituent cells. On the other hand either the massa cellularis reuniens (Kuhlenbeck, '24) or the subthalamic zone (Spatz, '27, '49; Kahle, '56 and Richter, '65) are held responsible for its primary origin. The expansion of the radiatio thalamostriatalis and of the other constituents of the capsula interna has the secondary effect of causing the alienation of the globus pallidus anlage from the matrix area where its constituent cells have originated. In this way this origin is obscured during the later phases of development. Another region that develops at the diencephalic side of the telodiencephalic boundary is the preoptic region. This region has a very close relationship to the pars inferior of the massa cellularis reuniens. During later stages the nucleus interstitialis of the stria terminalis is left at the place where this part of the massa cellularis reuniens used to be. By then the regio preoptica with its lateral, medial and periventricular nuclei is clearly located at the diencephalic side of the boundary that is formed by the torus transversus and torus hemisphaericus.

After this discussion of the caudal and rostral boundaries of the diencephalon we will now turn to a consideration of the constituent parts of the mammalian diencephalon as they were revealed by the ontogenetic approach that was followed in this study.

NEUROMERISM

Our investigation into the presence of neuromeres in the early phases of development of the neural tube has resulted in the affirmation of their existence from the neural plate stage onwards as was reported by a large number of authors (for a review of this literature the reader is referred to Bergquist, '52 and Vaage, '69). No sharp distinction between primary and secondary neuromeres (von Kupffer, '06) could be made. Neither could we distinguish between proneuromeres and neuromeres (Bergquist and Kallén, '54). The interneuromeric phases mentioned by the latter authors could not be detected in our material, notwithstanding the fact that we had a large number of closely spaced series at our disposal.

As far as the number of the prosencephalic neuromeres is concerned, a difference of opinion exists among the authors. In order to compare our findings readily to the data from the literature we prepared a number of diagrams in which a schematic representation of the various standpoints is given (see fig 66).

Within the prosencephalon an interneuromeric constriction ring was observed at the E_{11}-stage running from the velum transversum in a basal direction and crossing the midline, thus separating a prosomere A and a prosomere B (Von Baer, 1828; Mihalkovicz, 1877). The prosencephalon rostral to this boundary was called by Mihalkovicz prosencephalon primitivum, and by His (1893a) telencephalon. The latter used this constriction ring to define the boundary be-

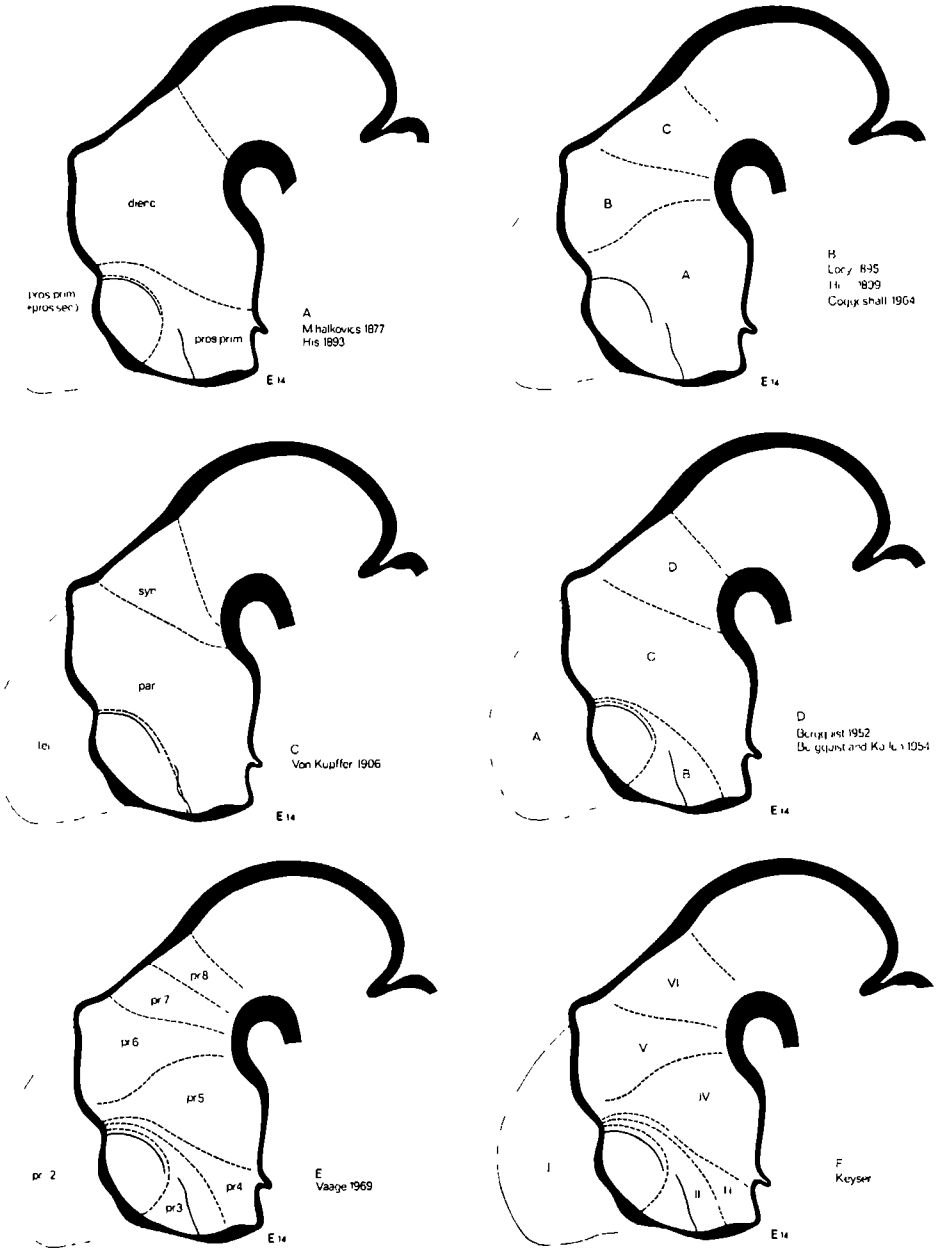

Fig 66 Schematic representation of the neuromeric subdivision of the prosencephalon as suggested by various authors; for a discussion, see text.

tween the telencephalon and the diencephalon in his proposals that were incorporated into the B.N.A. (1895). As was discussed above the boundary in question fades quickly and it is no wonder, therefore, that confusion prevails as to the place of its basal ending. His (1893[a]), Weber ('00), Kamon ('06) and Vaage ('69) were among the authors who placed it in between the infundibular and the mamillary anlagen. Johnston ('09), however, pointed to the misinterpretation of the recessus postopticus as the infundibulum in the early stages and he corrected the interpretation of this interprosomeric boundary. According to him, the constriction ring passes the basal midline in between the postoptic recess and the infundibulum.

After a careful scrutiny of our material we agreed with Johnston's interpretation and we therefore feel entitled to state that the constriction ring described by the other authors is materially the same as the one we observed to pass rostral to the infundibular anlage. The large prosomere rostral to this interneuromeric border (Mihalkovicz's prosencephalon primitivum or His's telencephalon) in later stages (E_{12} and later) was seen subdividing into three parts: the (secondary) telencephalon, the optic neuromere and the postoptic neuromere. Caudal to the interprosomeric border in E_{11} a parencephalon could be observed that in the E_{12}-stage is subdivided into an anterior and a posterior part. At $E_{12}1/_2$ a synencephalon could be distinguished rostral to the mesencephalic neuromeres (fig. 66f).

The opinion of Mihalkovicz (1877) and of His (1893[a]) as formulated above is represented in fig. 66a. In addition to the prosencephalon primitivum, Mihalkovicz discerned a prosencephalon secondarium corresponding to our telencephalon.

Von Kupffer distinguished three prosencephalic neuromeres, the telencephalon, the parencephalon and the synencephalon. The optic evagination was considered part of the parencephalon (fig. 66c).

Locy (1895), Hill (1899) and more recently Coggeshall ('64) distinguished three neuromeres within the prosencephalon: a large one rostral to the eventual external medullary layer of the thalamus and two neuromeres caudal to this layer, corresponding to the parencephalon posterius and to the synencephalon in our terminology (fig. 66b).

According to Bergquist and Källén ('54) the prosencephalon comprised four neuromeres; one telencephalic (neuromere I) and three diencephalic neuromeres (neuromere II, the optic neuromere; neuromere III, corresponding to our postoptic neuromere, parencephalon anterius and parencephalon posterius; and neuromere IV, corresponding to our synencephalon); cf. fig. 66d.

Vaage ('69) studied the chick embryo and discerned no less than eight prosencephalic neuromeres. Although his conclusions were obtained from avian material, we have ventured to transcribe his observations on our mammalian diagram (fig. 66e).

The question of the permanence of neuromeres in older stages could be answered definitely in the affirmative. Within the caudal part of the diencephalon the dorsal half of the interneuromeric borders between mesencephalon, synencephalon, parencephalon posterius and parencephalon anterius could be traced with great ease into the adult stage, as was described in chapter VII. Before these interneuromeric borders fade, fibre tracts and fibre laminae develop that make it possible to recognize these borders even after the development of a mantle layer. So the observations made by Palmgren ('24), Bergquist ('32, '52), Bergquist and Källén ('54) and Coggeshall ('64) receive affirmation from the results obtained in this study. The failure of other authors, as for instance Kuhlenbeck ('30, '31), to recognize these relationships can, in our opinion, be explained from the absence of early stages and from the lack of sufficiently closely spaced stages of development in their material.

THE VENTRICULAR SULCI

Since ventricular grooves play a prominent part in the ideas of subdivision of the brain, we studied the changes of the ventricular relief throughout development from the early neuromeric stages into the adult diencephalic configuration. It appeared that the ventricular relief, from being intimately related to neuromerism during earlier phases of development, becomes profoundly changed by the histogenetic events occurring in the wall of the neural tube. The interneuromeric crests show exhaustion of their matrix activity at an earlier stage than the central neuromeric regions. Consequently, the neuromeric situations are obliterated by the steadily thickening diencephalic wall. Eventually the interneuromeric eminences in some cases change into their opposites, as happens for instance to part of the syn-parencephalic crest which eventually participates in the formation of the sulcus diencephalicus dorsalis, and also to the interparencephalic crest which eventually becomes the transitory sulcus diencephalicus medius. These observations enabled us to take a stand in the discussion between the authors who looked upon the main diencephalic sulci as morphologically horizontal or longitudinal (His, 1888, 1893; Herrick, '10, '33; Kuhlenbeck, '30, '31), and those who considered them morphologically transverse structures (Haller, '29; Bergquist, '32; Bergquist and Källén, '54).

As can be seen in figure 32, part of the diencephalic sulci are of neuromeric origin; thus the sulcus intraencephalicus anterior, the sulcus lateralis infundibuli and the sulcus diencephalicus dorsalis can be considered, in part at least, a remnant of the optic, the anterior parencephalic, and the posterior parencephalic neuromeres respectively. They are therefore in part structures that originate transversely.

The sulcus diencephalicus ventralis and the sulcus diencephalicus basalis (cf. figs. 21, 23, 24) have a rather different origin. The former probably derives

from the tube-like form of the early diencephalon and from the difference in matrix activity between the basal and the dorsal parts of the wall of the neural tube. The latter results from the difference in development between the retarded median structures in the postoptic area and the more dorsally located progressive hypothalamic cell cord.

From this study of the ventricular relief it clearly emerges that the sulci are from multiple origin. Attention was focussed on the ventricular sulci by the debate concerning homology in the work of Herrick ('10, '33) and Kuhlenbeck ('27, '29, '30). These authors employed the sulci for the delimitation of mantle layer structures, and indeed, if rather closely related species are compared as far as the adult configuration is concerned, a similarity of the sulcal configuration can be observed. This observation was used by the authors just mentioned as an argument in favour of the reliability of the ventricular grooves as criteria in homologization.

From our study, however, it can be concluded that during ontogenesis the ventricular relief shows a continuously changing relationship with the mantle layer structures. Therefore, in an embryological study, ventricular grooves are of limited value for the definition of homologies. Sulci are entirely secondary to the histogenetic events. It is the histogenetic patterning within the wall of the neural tube that forms the primary feature which determines the eventual configuration of the brain.

When the validity of the sulcal pattern as a criterion of subdivision of the structures in the brain wall is compared to the validity of neuromerism, the latter turns out to be superior by far. Neuromerism is directly related to the histologic differentiation of the wall of the neural tube and in several places it clearly presses its mark upon the adult brain as was demonstrated in the chapter on mantle layer development.

THE SULCUS LIMITANS

After this discussion of the morphological nature of the diencephalic sulci and their value for the establishment of homologies, we will now turn to the sulcus limitans and set forth our view on the questions regarding the continuation and the ending of this sulcus within the prosencephalon.

The concept of the division of the lateral plates of the neural tube into a lamina basalis and a lamina alaris was introduced by His in 1888. It indicated a longitudinal division where the parts with a motor function were located in the lamina basalis and the parts with a sensory function in the lamina alaris. At the ventricular side of the neural tube a sulcus is observed, separating the basal and alar regions. This sulcus limitans (His, 1893[a]), therefore, runs a longitudinal course and unanimity of opinion exists on its occurrence within the deuterencephalic part of the brain. As far as the rostral continuity of the sulcus

limitans in the diencephalon is concerned, rather different views are advanced in the literature. Hochstetter ('19) doubts the existence of the sulcus limitans as such in this part of the brain. According to Kingsbury ('30) and Kuhlenbeck ('48) the sulcus limitans ends with a rostrodorsal convexity in the region of the mamillary recess. Others (His, 1893[a]; Johnston, '09; Streeter, '11) have described its ending in the preoptic recess, whereas Spatz ('27), Grünthal ('52), Kahle ('56) and Richter ('65) were able to trace the sulcus limitans through the foramen of Monro into the telencephalon.

In our opinion, the sulcus limitans problem is made up of three questions. The first question is whether a functional subdivision of the brain into a motor basal plate and a sensory alar plate can be made within the diencephalon. This question is beyond the scope of the present work and will not be answered here.

The second question is whether the mesencephalic sulcus limitans can be traced rostrally and whether this sulcus is continuous with a diencephalic longgitudinal sulcus. It proved to be difficult to follow the sulcus limitans of later stages rostrally beyond the synencephalic part of the brain, because the sulcus in question broadens in the synencephalic recess (= recessus metathalamicus) to such an extent as to stop being a sulcus at all. From the synencephalic recess no clear sulcus could be followed into the mamillary recess. The sulcus diencephalicus ventralis continues from the synencephalic recess in a rostral direction.

The third question concerns the place of the rostral ending of the sulcus diencephalicus ventralis, which a number of authors considered as the rostral part of the sulcus limitans (His, 1893[a]; Johnston, '09; Streeter, '11; Spatz, '27; Grunthal, '52; Kahle, '56; Richter, '65). From the sulcus diencephalicus ventralis a sulcus lateralis infundibuli is seen branching off in a basal direction (cf. fig. 23). The rostral ending of the sulcus diencephalicus ventralis appears to change according to the stage under consideration. At early stages, when the evaginating optic vesicles dominate the picture in the rostral part of the diencephalon (see for example E_{12}, fig. 17 and E_{13}, fig. 20), it was observed that the sulcus in question ends within the optic evagination. After the obliteration of the optic stalk, when the most rostral part of the sulcus diencephalicus ventralis and the primitive sulcus intraencephalicus anterior have fused, it could be observed that the sulcus diencephalicus ventralis bifurcates, one branch continuing its course into the preoptic recess and a second curving towards the foramen Monroi (cf. E_{15}, fig. 27). In still later stages the sulcus intraencephalicus anterior becomes less deep and eventually the sulcus diencephalicus ventralis seems to continue only into the foramen of Monro.

In our opinion the findings concerning the sulcus limitans as presented above explain the contradictory findings of the different authors. According to the stage under observation, the rostral ending of the sulcus diencephalicus ventralis is situated 1) in the preoptic recess, 2) in both the preoptic recess and the foramen of Monro and 3) in the foramen of Monro only

In the study of histogenesis of the diencephalon of the Chinese hamster we have concentrated on two aspects each one complementing the other, *i.e.*, the development of the matrix and the development of the mantle layer.

Throughout the diencephalon the histogenetic process follows the same sequence of proliferative activity, differentiation and matrix exhaustion. The rate of development, however, is different in each individual area. This heterochronous development of both matrix and mantle layer was studied and a characteristic pattern of heterochrony was observed.

The temporospatial patterning of matrix development within the diencephalon was studied previously by Kahle ('56) in the human embryo. In our study of matrix behaviour we benefited from the rather recently developed views on the natural history of the neuroepithelium (Sauer, '35a, '35b, '36, '37; Sauer and Walker, '59; Sauer and Chittenden, '59; Fujita, '63; Berry and Rogers, '65) that were not at Kahle's disposal. Employing these data we succeeded in subdividing the histogenetic process into nine different phases.

Two more points in which our study differs from Kahle's should be stressed at this juncture. First, we started our investigation at a considerably earlier developmental stage, *i.e.*, at the moment of closure of the anterior neuropore; this is quite important if the relationship between matrix development and neuromerism is the object of study. A second point of difference is that, contrary to Kahle, we did not take the His-Herrick-Kuhlenbeck scheme for granted. In our analysis accordingly the mapping of the matrix phases was accomplished with the developmental stage of the matrix as the sole criterion.

Our results demonstrated the same caudorostral and basodorsal gradients as were observed by Kahle; furthermore we were able to demonstrate in the earlier stages a definite progressive development of the interneuromeric borders which possess early mantle layer development. In these stages therefore a relationship between neuromerism and matrix activity must be assumed. At later stages of development it is not longer possible to demonstrate such a relationship within the more rostral parts of the diencephalon. Our conclusions from the study of later stages did not differ materially from those of Kahle and include the early differentiation of the matrix in the basal regions (prerubral tegmentum, regio subthalamica, tegmental cell cord, hypothalamic cell cord) and the exceptionally protracted development of the central postoptic, the preoptic and the epithalamic regions.

MANTLE LAYER DEVELOPMENT

The second aspect of histogenesis, *i.e.*, mantle layer development, has received considerable attention in the literature; in fact most studies on the development

of the mammalian diencephalon deal with the development of grisea and fibre tracts within the mantle layer. Therefore, the literature on diencephalic mantle layer development will be reviewed at this point.

An overall subdivision of the diencephalon in the human embryo was elaborated by His (1893[a]) who recognized four regions, three of them arranged longitudinally one above the other, *i.e.*, the hypothalamus, the thalamus and the epithalamus, the fourth being a caudally located subdivision, the metathalamus.

On the basis of a comparative study in lower vertebrates, Herrick ('10) subdivided the thalamus of His into a thalamus dorsalis and a thalamus ventralis. Hence he arrived at a division of the diencephalon into four longitudinal regions, epithalamus, thalamus dorsalis, thalamus ventralis and hypothalamus. According to Herrick these subdivisions are separated from each other by ventricular sulci, *i.e.*, the sulcus dorsalis thalami, sulcus medius thalami and sulcus ventralis thalami.

The conclusions reached by Herrick ('10) were extended to the entire vertebrate kingdom by Kuhlenbeck ('27). In a series of investigations ('29, '30, '31, '36, '54) this author worked out a 'Bauplan' of the vertebrate diencephalon in which the four diencephalic stories of Herrick formed the fundamental units or 'Grundbestandteile'. These were defined by the ventricular boundary sulci enumerated above. Although originally derived from the adult anamniote's brain, the pattern of four stories bounded by three ventricular sulci was found by Kuhlenbeck to be a distinct feature, in certain embryonic stages at least, of the diencephalon of the amniote brain too, thus adding to its credibility as a fundamental characteristic of the diencephalic part of the brain. Most authors studying the ontogenesis of the mammalian diencephalon have produced evidence sustaining the His-Herrick-Kuhlenbeck scheme, and their findings will be reviewed briefly. Pioneering studies in this particular field were those of Bianchi ('09) and Droogleever Fortuyn ('12) on the development of the rabbit's diencephalon.

Bianchi, starting his study at the 15 mm stage (which, judging from his illustrations, is comparable with the E_{14} Chinese hamster embryo), confirmed the existence of His's divisions of the diencephalon in the rabbit embryo and gave an amply illustrated survey of the development of the thalamus. At this juncture we should like to call attention to the fact that the point of departure for Bianchi's study was chosen at a stage where the neuromeric pattern is fading already. It cannot be stressed too much that, in order to detect the neuromeric pattern and to reveal its impact on the adult configuration of the brain, one has to start one's study at the very origin of the neural tube and that closely spaced series are required in order to follow the developmental process. Droogleever Fortuyn ('12) also studied the development of the rabbit's diencephalon up from a comparable stage and made his results more accessible by the use of the graphic reconstruction technique. His conclusions pointed to the existence of the four

longitudinal diencephalic regions as proposed by Herrick, separated, at some embryonic stages at least, by ventricular sulci. The boundary between dorsal thalamus and ventral thalamus was recognized on the basis of a difference in matrix structure. Droogleever Fortuyn interpreted the ventral thalamus as the anlage of the eventual ventrobasal thalamic complex. The study of Kuhlenbeck on the ontogenesis of the mammalian diencephalon ('30) fell a victim to the same misinterpretation of the pars ventralis thalami. This was corrected, however, in 1933, by a student of Kuhlenbeck, Miura, who, once again, studied the development of the rabbit's brain. He confirmed the existence of both Herrick's longitudinal zones and the ventricular sulci in between, for the mammalian diencephalon Miura did not include the disputed derivation of the corpus geniculatum mediale in his study but his results were mentioned in a separate paper by Kuhlenbeck ('34) who concluded that the corpus geniculatum mediale was a forthright diencephalic derivative.

Rose ('42) re-studied the development of the rabbit's diencephalon and concluded to the existence of two main divisions within this part of the brain, the dorsal division forming the thalamus, the ventral division the whole hypothalamus. The primordial thalamic area differentiates according to his observations into three functionally different constituents: 1. an independent dorsalmost thalamic area consisting of cells which do not degenerate after cortical ablation and corresponding to the epithalamus; 2. a 'telencephalon dependent' thalamus, developing into nuclear complexes that degenerate without exception after cortex ablation and corresponding to the pars dorsalis thalami; and 3. the ventral 'connecting' thalamus corresponding to the pars ventralis thalami which is traversed by the thalamic radiations and forms the connection with the hypothalamus and with the telencephalon. The nomenclature used in this subdivision of the thalamic part of the diencephalon is clearly influenced by the relationships of the thalamic nuclei discovered in the thirties by means of degeneration experiments (see *i.a.* Clark and Boggon, '32; Walker, '38). From a morphological point of view the terminology employed is rather debatable. Within the hypothalamic cell plate, Rose discerned a dorsal or subthalamic area, a central hypothalamic area and an anterolateral hypothalamic area Essentially Rose confirmed the His-Herrick subdivision of the diencephalon into four zones, *i.e.*, the independent or epithalamic region, the dependent or dorsal thalamic region, the connecting or ventral thalamic region and the hypothalamus.

Niimi, Harada, Kusaka and Kishi ('61) studied the development of the diencephalon in the mouse and started their description with the recognition of Herrick's four columns in the ten to twelve days brain tube. In their illustrations of these early stages interneuromeric crests can be discerned, but, probably because of the lack of reconstructions in their work, they failed to recognize them as such. A striking feature of the regional description of the development of the four stories by these authors was the inclusion of the pretectum within the

epithalamic zone.

The results of the studies mentioned above were obtained mainly on lower mammals. They were confirmed by Gilbert ('35) who studied the development of the human diencephalon. Her study was illustrated with outline reconstructions from sagittally sectioned series; similar reconstructions were used in the paper of Papez ('40) on human hypothalamic development. Kahle ('56), studied the role of the matrix phases in the human diencephalon. It is worth noting that for the interpretation of the spatial relationships of the matrix areas Kahle relied to a large extent on the same reconstructions from Papez's study. More elaborate studies on the development of the human dorsal thalamus were published by Cooper ('50), by Dekaban ('54) and by Fernández-Ortega ('68). These studies included as their earliest stages embryos comparable to the E_{13}-stage of the Chinese hamster embryo.

All the studies enumerated thus far concur in the confirmation of the existence, at embryonic stages, of the diencephalic stories elaborated by the His-Herrick-Kuhlenbeck school. All of the authors mentioned, however, leave the configuration of the neural tube which is present before the appearance of the four floors out of consideration, or, if an allusion to such a pre-existent configuration is made, they all deny any demonstrable relationship between the components of that early neural tube and the eventual four sulci-bounded diencephalic floors. In other words, the subdivision of the diencephalon as originally derived from rather advanced embryonic stages (His, 1895), is recognized in the adult brain and then traced back, sometimes far beyond the stage it was derived from. Surveying the work of these authors, we got the strong impression that they have attempted to force their observations into a preconceived framework, namely the four story diencephalon, rather than derive criteria from early stages for a subdivision of the adult brain. A number of authors, however, refused to follow this beaten track.

As early as 1929, Haller called attention to the presence of ventricular sulci corresponding with neuromeres early in development. He also pointed out that a definite relationship exists between this early relief and the later diencephalic sulci, described as longitudinal grooves by Herrick ('10) and Kuhlenbeck ('29). He concluded to a morphologically transversal position of these sulci and thereby also of the diencephalic stories in between.

The validity of the His-Herrick-Kuhlenbeck scheme was also seriously questioned by the exhaustive study of Bergquist ('32) on the development of the diencephalon in anamnia. Bergquist demonstrated that the neural tube wall of early stages possesses regions with a high mitotic density. These proliferation centres showed a tendency of causing a bulging outward of the neuroepithelium, which was accompanied by the formation of ventricular grooves. The proliferation centres give origin to cerebral nuclei by a process of differentiation of cells

and by their subsequent migration into the periphery of the wall of the neural tube. Bergquist considered them to be fundamental units or 'Grundgebiete' that together form the earliest indication of the adult configuration. Extensive embryological studies, partly carried out in cooperation with Källén, revealed that the 'Grundgebiete' or migration areas are the constituents of a third generation of neuromeres as discerned by Bergquist and Källén ('54), *i.e.*, the so-called transversal bands (Källén, '54) or postneuromeres (Bergquist and Källén, '55). This generation is preceded by the proneuromeres and the neuromeres of Bergquist and Källén's denomination as has been explained in chapter IV. Both migration areas and postneuromeres develop from neuromeres through an interneuromeric phase II, but, as stated by Bergquist and Källén ('53), the neuromeres become indistinct when the migration areas develop and this fact makes the comparison between the areas and the neuromeres difficult. In addition, the neuromeres in the prosencephalic part of the brain are soon obscured by the evagination processes taking place Furthermore it is difficult to find a stage where both neuromeres and postneuromeres are developed in this part of the brain, since the change from neuromeres into postneuromeres is said to take place very rapidly. Nevertheless the transversal bands (*i.e.*, the postneuromeres) present in the prosencephalon have been shown to correspond to the neuromeres in a certain way (see below).

The dissolution of the neuromeres and the formation of migration areas is accompanied by the appearance of a longitudinal banding caused by a basodorsal heterochrony gradient. These longitudinal bands correspond to the functional longitudinal columns discussed by Gaskell (1889), Strong (1895) and Herrick (1899). Thus a chequered pattern originates, the migration areas constituting both longitudinal columns which pass the interneuromeric borders, and transversal bands which correspond to the third generation of neuromeres. Bergquist and Källén ('53b) described the relations of the developed migration areas to both neuromeres and postneuromeres. Within neuromere I an area dorsalis telencephali and an area ventralis telencephali are observed; within neuromere II an area optica and an area rostralis thalami are seen; neuromere III develops into an area medialis thalami, an area caudalis thalami, the hypothalamic areas and the area tuberculi posterioris; neuromere IV consists of the area commissurae posterioris and the area fasciculi longitudinalis medialis.

The recognition of postneuromeres or transversal bands by Bergquist and Källén within the prosencephalon seems to be founded in part on the development of only two longitudinal columns within this part of the brain, a dorsolateral column embracing all dorsal diencephalic migration areas and the whole of the telencephalon, and a ventrolateral one, consisting of the hypothalamic and the more caudal prosencephalic areas. The postneuromeres are arranged perpendicular to these longitudinal columns; the first postneuromere consists of an area ventralis telencephali and an area optica dorsally, corresponding to an area

rostralis hypothalami basally. The second postneuromere is composed of an area dorsalis telencephali and an area rostralis thalami dorsally, corresponding to an area intermedia and an area caudalis hypothalami basally. The third postneuromere consists of an area medialis and an area caudalis thalami dorsally, that correspond to an area tuberculi posterioris basally. The fourth postneuromere comprises an area commissurae posterioris dorsally and an area fasciculi longitudinalis medialis basally.

The subdivision of the lateral diencephalic wall thus reached by Bergquist and Källén into Grundgebiete or migration areas, is obtained by the application of criteria that must enable the investigator to characterize the different areas and to recognize them in different species and in different specimina. When the literature on this subject is searched for a definition of the term migration area, emphasis seems to be laid more on what is in the centre of the area than on the description of its boundaries. Bergquist ('32, p. 66) states: "In den erwähnten Rekonstruktionen sind indessen eine Anzahl Grenzen von der Ventrikelfläche anliegenden Zellengebieten eingezeichnet worden. Diese Grenzen sind aber in Wirklichkeit nicht an den Ventrikelfläche markiert, sondern werden nur auf denselbe projiziert gedacht. Denjenigen Teil der Ventrikelfläche der einem derartigen Zellengebiet entspricht, ob die Fläche nun von Furchen begrenzt ist oder diese auf die Fläche projiziert gedacht werden, nenne ich Area ... Unter einem Zellengebiet ... wird im folgenden ausschliesslich eine zytoarchitektonische Zelleneinheit verstanden". On page 73 Bergquist continues: "Ontogenetisch treten diese Grundgebiete gewöhnlich sehr früh auf. Ihre Abgrenzung erscheint da bisweilen nicht überall im einzelnen scharf, wenn sie auch in ihrer Gesamtheit unverkennbar ist. Später sind jedoch die Grenzen der Grundgebiete gewöhnlich deutlich ausgebildet. Von grossem Interesse war es nun zu finden, dass die Grundgebiete, praktisch genommen, stets mehr oder weniger um Proliferationsfurchen herumliegen, in deren Umgebung die Zellteilung am lebhaftesten zu sein scheint".

In the large number of publications published by Bergquist and Källén in the fifties, a more exact definition was not given and a migration area was characterized repeatedly as the ventricular area corresponding to the region within which the first migrations take place. This migration is the first sign of nuclear differentiation. It is preceded by a crowding of mitoses in that particular area (*i.e.*, proliferation) and usually also by the formation of a groove (a proliferation furrow), formed ventricularly in the middle of the migration area (Källén, '51; Bergquist and Källén, '54).

The neuromere-based approach to neuroembryology, as summarized above, was succesfully pursued by the Swedish authors through the whole series of vertebrates. Studies, which are pertinent more in particular to the mammalian diencephalon, were published by Bergquist ('54a, b). Five neuromeres were discerned within the diencephalon by Bergquist. His neuromere II corresponds to

our optic neuromere His neuromere III corresponds to the postoptic neuromere, the parencephalon anterius and the parencephalon posterius of our terminology His neuromere IV corresponds to the synencephalon of the present study

On account of the fact that the studies of Bergquist and Kallen are the only ones that have a scope comparable to our study we ventured to make a comparison between the subdivision of the diencephalon into migration areas as accomplished by Bergquist and the ontogenetic units discerned in the present study As far as the occurrence of migration areas is concerned we confirmed Bergquist's subdivision of the diencephalon into a number of basal regions and a number of dorsal regions As to the exact delimitation of these ontogenetic units in the Chinese hamster our opinions diverged The area commissurae posterioris of Bergquist (B) corresponds to our regio pretectalis The area of the fasciculus longitudinalis medialis (B) is our synencephalic prerubral tegmentum The area tuberculi posterioris (B) roughly corresponds to our regio subthalamica The area caudalis thalami (B) and the area medialis thalami (B) correspond approximately to our pars dorsalis thalami and pars ventralis thalami The area rostralis thalami (B) is situated at the place where we indicated the subthalamic cell cord Bergquist s area caudalis hypothalami and the area intermedia hypothalami are located at the site of our regio mamillaris, and the area rostralis hypothalami (B) has a number of characteristics in common with our regio postoptica These correspondences, however, are only approximate and for a detailed comparison the reader is referred to table IV where we have made an attempt to bring together in one table the derivatives of the various areas and regions discussed above

Rather independent positions are held by Stroer (56) and Coggeshall (61) who both studied the development of the diencephalon of the rat

Stroer gave a description of the development of the diencephalon of the rat from the stage when the neural tube is just closed to the 16 days old embryo on the basis of some graphic reconstructions In early stages he found a transverse neuromere derived subdivision of the diencephalon The neuromeric cavities become transformed into grooves and these grooves are redirected by the developmental process and eventually they subdivide the diencephalon into the His Herrick Kuhlenbeck stories The material, on which Stroer based his study, however, was too restricted to enable the author to observe the relationships between the primary ontogenetic units in the ventricular wall and the morphogenetic changes of the ventricular surface

Coggeshall (64), who also studied rat material, recognized the neuromeric subdivision in rather advanced embryos and even succeeded in outlining the derivatives of prosencephalic neuromeres in the adult rat brain In the interparencephalic border he observed the development of the external medullary lamina of the thalamus, caudal to the parencephalon posterius he described the

development of the fasciculus retroflexus and an associated fibre layer, and in the syn-mesencephalic boundary he discerned the fibres of the commissura posterior. The presence of these persisting fibre structures enabled him to follow the boundaries in question into their adult position. Our results contain a full affirmation of the observations of Coggeshall in this respect.

This brings us to a discussion of the part played by fibre connections in homology questions. The fact that the same hodologic relationships of cerebral nuclei in different species only imply the existence of analogy and not necessarily lead to the homology of the grisea in question, may be supposed to be common knowledge. As the detailed hodogenesis of the diencephalon was outside the scope of our study, we shall limit this discussion to the relations between the developing fibre tracts and the criteria of subdivision of the brain that were investigated in the analytical part of this study.

The keystone for tracing the interneuromeric borders in the adult brain is the development of fibre structures in these boundaries. This phenomenon gives rise to the following question: Is the morphogenetic process responsible for the topogenesis of the interneuromeric fibre tracts or are the fibre tracts responsible for the origin of structures like the interneuromeric crests? Blechschmidt and Arndt ('67) studied this same question. They concluded that the growth of nerve cell extensions is favoured by the presence of certain histologic conditions. These conditions are met in a zone in which a local pressure is exerted as is the case in between two adjacent proliferation centres, and from their observations they concluded that the direction of growth of the nerve fibres in this zone of histological change is perpendicular to the direction of the pressure exerted on the zone in question. The development of the fibre tracts in the interneuromeric crests was thus attributed to the patterning of the proliferation process. In our material the presence of interneuromeric constrictions was also observed to precede the development of fibre tracts at corresponding sites in later stages. We therefore concluded that in the case of the location of the boundary-forming fibre structures (as, for instance, the commissura posterior and the fasciculus retroflexus) the histogenetic process and the patterning of the same are the primary features. After their emergence, however, the fibre tracts in question do exert a developmental kinetic influence on the further morphogenesis of the brain. They provide a kind of skeleton which determines in part the future shape of the growing brain.

A quite different question concerning the relationships between cerebral nuclei and nerve fibres is whether the ingrowth of axons in a particular subdivision of the brain influences the histogenetic process in that region and whether the ingrowth of fibres causes the layers of migrated cells to become subdivided into nuclei. If they could be demonstrated, such an influence and subdivision would

Bergquist 1954		the present study
area optica — pars rostralis	nucleus preopticus lateralis nucleus preopticus medialis nucleus preopticus periventricularis	*regio preoptica*
area optica — pars caudalis	nucleus supraopticus nucleus paraventricularis nucleus anterior hypothalami nucleus suprachiasmaticus nucleus supraopticus diffusus	*regio supraoptica*
area rostralis hypothalami	nucleus lateralis hypothalami nucleus tuberis lateralis nucleus ventromedialis	*regio postoptica*
area intermedia hypothalami — pars rostralis	nucleus dorsomedialis nucleus premamillaris	*regio postoptica*
area intermedia hypothalami — pars caudalis	nucleus mamillaris medialis nucleus mamillaris lateralis nucleus intercallatus	*regio mamillaris*
area caudalis hypothalami	nucleus supramamillaris nucleus posterior hypothalami	*regio subthalamica*
area tuberculi posterioris	corpus subthalamicum Luysi	*regio subthalamica*
area medialis thalami pars caudalis	fields of Forel	*regio subthalamica*
area rostralis thalami (in part)	zona incerta (in part)	*regio subthalamica*
area fasciculi longitudinalis medialis		*prerubral tegmentum*

Bergquist 1954		the present study
area intercalata	nucleus praethalamicus	*pars ventralis thalami*
area rostralis thalami (in part)	zona incerta (in part)	
area medialis thalami	nucleus reticularis thalami	
	nucleus medialis thalami	*pars dorsalis thalami*
area caudalis thalami	ventrobasal thalamic complex	
	nucleus lateralis posterior	
	nucleus habenularis lateralis	*epithalamus*
	nucleus habenularis medialis	
area commissurae posterioris	pretectal nuclei	*regio pretectalis*
	nucleus posterior thalami	

Table IV. A comparison between the subdivision of the mammalian diencephalon in migration areas as achieved by Bergquist (1954) and the subdivision as derived from the present study.

be clearly of importance for the establishing of homologies (Kallen, '54). This approach to homology was pursued by Senn ('68) who observed the presence of alternating fibre- and cell layers throughout the reptilian diencephalon and mesencephalon. In our study no indications of the existence of such a pattern in the mammalian diencephalon were observed.

THE MORPHOLOGICAL PLAN OF THE DIENCEPHALON OF THE CHINESE HAMSTER

The criteria used for the grouping of several nuclei into one subdivision of the diencephalon in the literature discussed above, are derived from various sources. To conclude this thematic discussion we shall now present a succinct description of the diencephalic Bauplan as it was revealed by the analysis of the development of the brain of the Chinese hamster. In our study we tried to demonstrate that there is a natural self-subdivision of the embryonic brain. It was found that caudal to the parencephalon anterius a neuromeric pattern could

be followed up to the adult stage. Rostral to the interparencephalic border a number of ontogenetic units was observed, which do not correspond to the neuromeres; they manifest themselves in the E_{14}-stage, and could be followed readily through later stages.

In order to analyse this rather complex situation a number of diagrams was designed (fig. 67). In figure 67a the interneuromeric borders are represented as they appear in the E_{14}-stage. Rostral to the interparencephalic border the parencephalon anterius is located. Its rostral boundary is indicated by the broken line extending from the velum transversum dorsally to the rostral limit of the infundibulum basally. This border coincides with the original border between parencephalon and prosencephalon primitivum (in the sense of His, 1888, and Mihalkovicz, 1877).

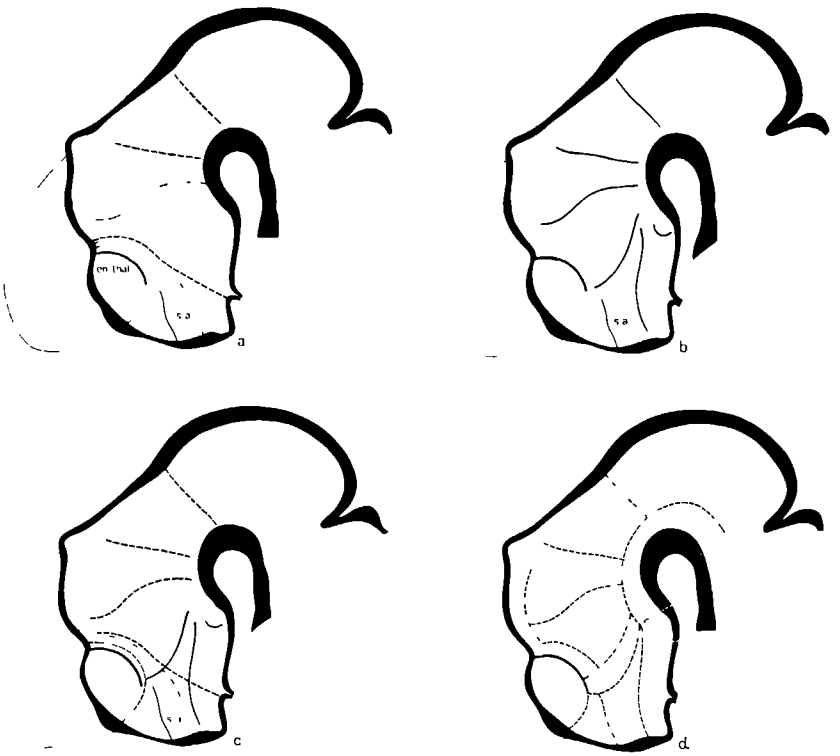

Fig 67 Schematic representation of the results of the analysis of the structural pattern in the diencephalon of the Chinese hamster, for a discussion, see text

Within this prosencephalon primitivum a prosencephalon secundarium or telencephalon and an optic neuromere have developed, separated by the interneuromeric border that was described in chapter IV and that is characterized by

a progressive development of the mantle layer. Within the optic neuromere the increasing rostrally directed relative displacement of the optic stalks effects the appearance of a separate area which was accepted by Vaage ('69) as a separate neuromere and which we called the postoptic neuromere.

Further, in fig. 67b, it is shown that from the progressive tegmental area a number of progressive differentiating areas develop which in the region caudal to the parencephalon anterius coincide with the interneuromeric crests. Rostral to the interparencephalic crest a number of differentiation strips is represented, not coinciding with the interneuromeric borders, but on the contrary, crossing them. In fig. 67c these rostralmost strips of differentiation are illustrated once more, together with the interneuromeric crests.

Thus, rostral to the interparencephalic border a complex situation is met. The number of distinguishable areas in this early E_{14}-stage (illustrated in fig. 67d) can be followed throughout development. Consequently we are in a position to state that the adult configuration of the diencephalon already is established in the E_{14}-stage, and we are of the opinion that the areas present at this stage can be considered to be the basic structural elements or entities of the diencephalon of the Chinese hamster. Together they constitute a skeleton which can serve as a reference scheme for this particular species, and its presence may be looked for in other vertebrates as well.

THE MANTLE LAYER DERIVATIVES IN THEIR RELATION TO THE SUBDIVISIONS OF THE DIENCEPHALON

In the first part of this chapter which aims at a synthesis, the developmental phenomena were studied thematically and our findings were compared with the data from the literature. In the ensuing second part of this chapter a regional survey of the mantle layer and its derivatives will be presented. This discussion offers an opportunity to compare the results obtained in the present study on the subdivision of the mantle layer to those of other authors and to evaluate the justification of the various subdivisions of the diencephalon into regions and zones as found in the literature. In order to facilitate orientation five transverse sections of the adult diencephalon are presented (see fig. 68).

Epithalamus

Few authors, who have studied the development of the diencephalon, have given the epithalamus more than an occasional glance. Yet, unfortunately, no consensus of opinion exists as to the extent of this part of the diencephalon. Most authors (see, for example, Kuhlenbeck, '54; Zeman and Innes, '63) use this term to indicate the structures that connect the pars dorsalis thalami and the chorioideal roof of the third ventricle together with the median pineal body or

epiphysis. Both its rostral and its caudal constituents, however, are the object of much debate.

At rostral levels this dorsalmost part of the diencephalon is represented by the stria medullaris fibres which link the habenular complex and basal telencephalic structures and which pass through the eminentia thalami. In this eminence the stria medullaris is accompanied by a nucleus paraventricularis anterior and a nucleus parataenialis which, according to some authors (among them J. E. Rose, '42; Dekaban, '54; and Kahle, '56) are both to be considered rostrally displaced components of the epithalamic complex. At more caudal levels a nucleus habenularis medialis and a nucleus habenularis lateralis can be discerned. According to J. E. Rose ('42) who introduced a functional subdivision of the dorsal diencephalic regions, the prebigeminal or pretectal group of nuclei, the adjacent nucleus paraventricularis posterior and the nucleus suprageniculatus pretectalis, have to be counted among the nuclei constituting his 'cortically independent' thalamus; this 'independent' thalamus in his opinion also comprises the epithalamic nuclei enumerated above. According to Coggeshall ('64) the epithalamus is located between the posterior thalamic septum (which develops in the syn-parencephalic border-zone in connection with the fasciculus retroflexus) and the posterior commissure and so embraces the whole of the pretectal group of nuclei plus the habenular nuclei. Thus the epithalamus as described by Rose and Coggeshall is considerably larger than the epithalamus as defined by most other authors. In this matter Coggeshall relied upon Herrick ('10) who, in the amphibian brain, defined the epithalamic floor as comprising the habenular nuclei plus the pars intercalaris diencephali. The latter part of the diencephalon is the equivalent of the dorsal part of the synencephalon of our study, in which the pretectal group of nuclei develops. Many authors, however, consider this part of the brain to belong to the thalamus dorsalis (see for example Gurdjian, '27; Kuhlenbeck, '54; Holmes, '53; Hess, '54).

It appeared from our study that the epithalamic region is relatively large at the earlier stages of ontogenesis. It extends from the velum transversum to the pars precommissuralis of the pretectum, lying in between the pars dorsalis thalami and the plexus chorioideus ventriculi tertii. In later stages of development this relative preponderance of the epithalamus gradually decreases as the pars dorsalis thalami enlarges. Simultaneously the epiphysis, which originates in the midline, halfway the dorsal wall of the parencephalon posterius, is displaced backwards; eventually it becomes located near the syn-parencephalic border. From our study it could not be decided whether this displacement should be considered an absolute shift or as a relative translocation, caused by the disproportionate development of adjacent parts of the diencephalon. As regards the nucleus parataenialis and nucleus paraventricularis anterior, we consider it likely that both of these cell masses are derivatives from the dorsalmost, *i.e.*, epithalamic subdivision of the parencephalon posterius. No valid reason was

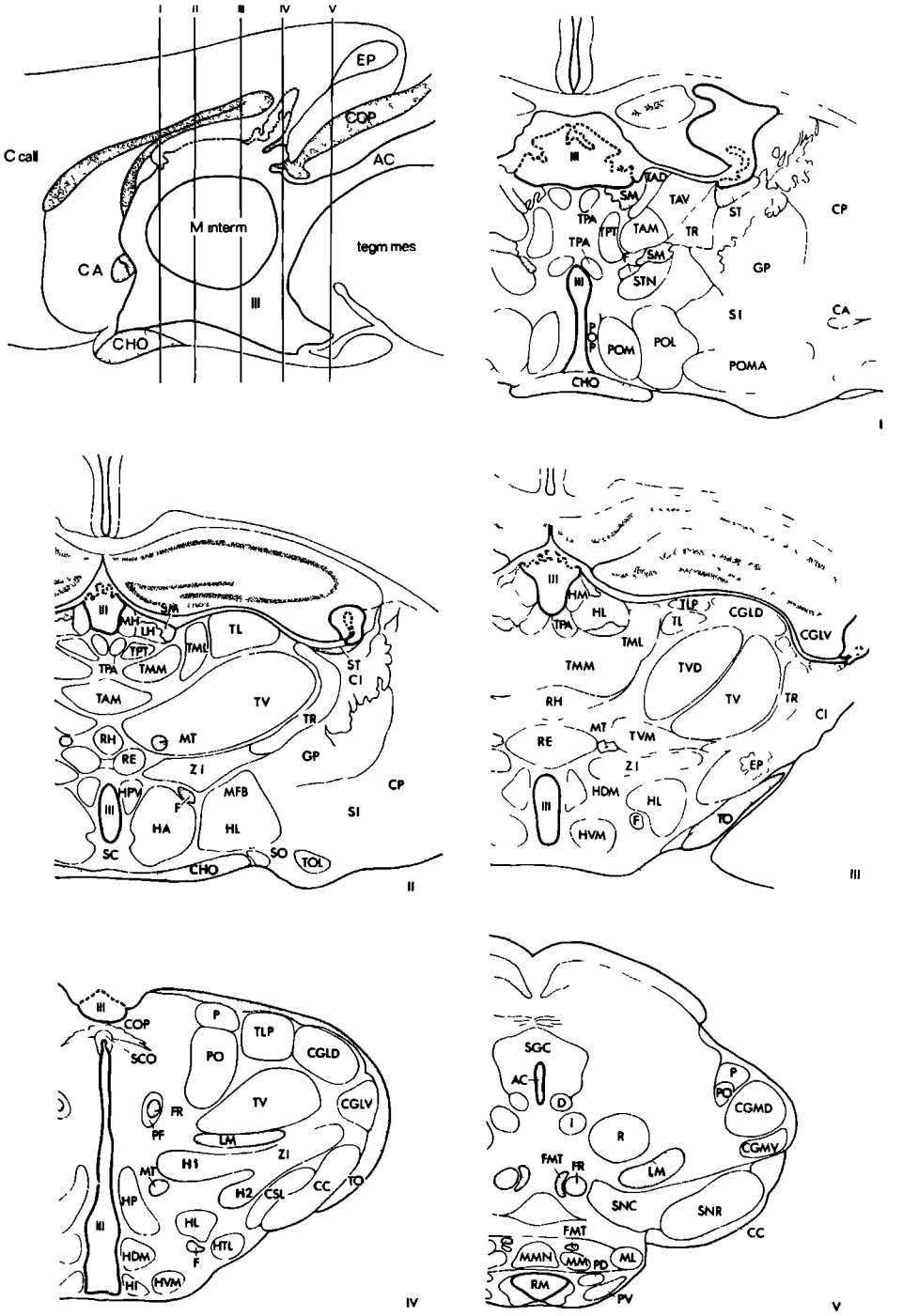

Fig. 68 Atlas of the diencephalon of the adult Chinese hamster as observed in the transversely sectioned series

found to include the pretectal nuclei within the epithalamus as they clearly were seen to originate within the pars dorsalis synencephali.

Pars dorsalis thalami

We used the term pars dorsalis thalami in order to indicate the fact that this region comprises only part of what a good many of authors have called 'thalamus' (Bianchi, '09; Rose, '42; Hess, '54; Holmes, '53).

An elaborate discussion of the different components of the pars dorsalis thalami is not our aim; we will confine ourselves to the discussion of the pars dorsalis as it presents itself in the early embryonic stages of development; furthermore we intend to give a clearcut picture of its boundaries.

The parencephalon posterius shows, in its dorsal part at least, a very clear delimitation from the adjacent parts of the diencephalon. In the foregoing paragraph a description was given of the epithalamus, the dorsalmost part of the neuromere in question The pars dorsalis thalami develops in a basal position to the epithalamus. It can be delimited from the caudally placed pars dorsalis synencephali by a thalamic septum (Coggeshall, '64), and from the rostrally placed parencephalon anterius by the zona limitans intrathalamica which develops into the lamina medullaris externa of later stages. Basally this pars dorsalis thalami borders on the subthalamic region, and the ventricular border between the two regions is marked in later stages by the sulcus diencephalicus ventralis The increasing curvatures of the various boundary-planes mentioned above then obscure the originally rather simple relationships.

The fact that the posterior thalamic septum, which develops in the syn-parencephalic boundary zone, becomes indistinct in the later phases of development, makes it understandable that most authors did not recognize it in the adult brain. Thus a number of synencephalic nuclei is commonly included in the pars dorsalis thalami as a posterior group of nuclei. These are: the nucleus posterior thalami (Cajal, '11), the nucleus pretectalis, the area pretectalis and the nuclei commissurae posterioris (Gurdjian, '27; Kuhlenbeck, '54). In our study the combination of an embryological approach and the study of adult series sectioned in various directions, enabled us to follow this syn-parencephalic border throughout its development. In the earlier phases of development the syn-parencephalic boundary is accentuated by the appearance of the fasciculus retroflexus. It is the presence of this bundle which makes the boundary in question recognizable during later stages. The dorsal half of the syn-parencephalic border is characterized by its rostrally convex shape, which stands out very clearly in the adult in series sectioned in the so-called horizontal plane. Therefore it is our firm conviction that the inclusion of the derivatives of the pars dorsalis synencephali into the pars dorsalis thalami is not warranted because of the confusion that is

caused thereby from the point of view of developmental and of comparative neuroanatomy.

The corpus geniculatum mediale develops at the parencephalic side of the border in question. Its relatively caudal position at later stages is accounted for by the fact that it is the earliest differentiating nucleus within the pars dorsalis thalami and therefore becomes firmly anchored to the tegmental structures which also differentiate early and which are situated caudobasal to it. The other dorsal thalamic constituents, which differentiate at a later stage, retain, so to speak, enough plasticity to be moulded by the morphogenetic movements into relatively more rostral positions. The dorsal part of the corpus geniculatum laterale develops in a position rostrodorsal to the corpus geniculatum mediale. In the brain of the Chinese hamster there is more justification to speak of an anterior and a posterior position of the geniculate bodies than to use the accepted designation as lateral and medial structures, as was remarked already by Forel (1872).

Pars ventralis thalami

In the present study the term pars ventralis thalami is employed to indicate the dorsal part of the parencephalon anterius. As such the pars ventralis thalami is located rostral to the pars dorsalis thalami. It comprises the nucleus reticularis thalami and part of the zona incerta. Kuhlenbeck ('48, '54) was of the opinion that the ventral parts of both the corpus geniculatum laterale and the corpus geniculatum mediale belong to this division of the brain. As far as the existence of a pars ventralis of the corpus geniculatum mediale is concerned, no unanimity of opinion exists in the literature. This structure was described by Le Gros Clark ('33) and Kuhlenbeck ('35, '54). In most descriptions of the rodent brain, however, no mention is made of this nucleus (Gurdjian, '27; Holmes, '53; Hess, '54). Presumably the corpus geniculatum mediale pars ventralis of the denomination mentioned above is considered a caudal extension of the zona incerta by a number of authors (see, *e.g.*, Schneider, '70). In our analysis of the development of the mantle layer we presented evidence for the derivation of the ventral parts of the geniculate bodies from the regio subthalamica which constitutes the basal half of the parencephalon.

In early development the pars ventralis thalami has a relatively large ventricular surface (cf. E_{14}, fig. 25 and E_{16}, fig. 28). The reduction of this surface during subsequent stages is probably effected by the disproportionate development of the pars dorsalis thalami. In the adult brain hardly any ventricular representation can be found of this part of the diencephalon because the sulci, which in later embryonic stages form the limits of the pars ventralis thalami (*i.e.*, the sulcus diencephalicus medius and the sulcus diencephalicus ventralis), fuse to become eventually the sulcus hypothalamicus (s. Monroi).

In this study the term "thalamus ventralis" has been avoided since, in our opinion, it has been employed for structures the homology of which has not yet been ascertained sufficiently. Herrick ('10, '33) employed this term to designate a largely motor zone in the amphibian brain, whereas Kuhlenbeck ('27) homologized this "thalamus ventralis" with the area in the adult amniote brain that is located between the sulcus diencephalicus medius and the sulcus diencephalicus ventralis leaving its functional significance out of consideration. In our opinion the data from the literature concerning this problem are contradictory; therefore we decided to use the term 'pars ventralis thalami', thus following Kahle ('56) who discussed this problem in some detail.

So far only those diencephalic regions have been discussed that are located in the dorsal parts of the brain tube. The pars dorsalis synencephali or the pretectal group of nuclei have been delimited from both the epithalamus and the pars dorsalis thalami. Rostral to epithalamus and pars dorsalis thalami, the pars ventralis thalami was observed. In the following section of this survey the basal counterparts of the four regions just mentioned will be considered.

Prerubral Tegmentum

The prerubral tegmentum derives from the pars basalis synencephali. In this area a nucleus interstitialis (Cajal) and a nucleus of Darkschewitsch have been identified in a juxtamedian position. These nuclei are seen developing from the tegmental cell cord which extends rostrally beyond the syn-parencephalic border.

The basal synencephalic structures are supplemented from a mesencephalic tegmental source; here, amidst the early differentiating structures, in the midline a production of cells continues in the region of the fovea isthmi. These cells pierce the tegmentum in the median plane and, having reached the basal external surface, they fan out in a lateral direction, thus providing material for the eventual nucleus interpeduncularis, the prerubral field of Tsai and the substantia nigra; the latter two structures, in part at least, are eventually located synencephalically although their source of origin is located ventricularly in the region of the fovea isthmi.

Regio subthalamica

Rostral to the synencephalon and basal to both pars dorsalis thalami and pars ventralis thalami the regio subthalamica develops. This region is characterized by its early differentiation. No clear boundaries can be indicated but the fasciculus retroflexus can serve as a landmark for its caudal limit. Ventricularly the sulcus diencephalicus ventralis marks the dorsal boundary. Here, more than in any other brain region, it becomes clear that without taking into account the

histogenesis of the area under consideration, no sensible boundaries can be found.

When histogenesis is considered three different zones can be distinguished within the regio subthalamica. In juxtaposition to the ventricular surface early differentiating cells are observed which develop from the rostral extension of the tegmental cell cord. Laterobasal to this nucleus posterior hypothalami a formation is observed consisting of longitudinal fibre tracts and interstitial cells, which shows a reticular appearance. The two collections of neurons mentioned thus far derive from the most dorsal matrix of the subthalamic region. A third group of grisea derives from the matrix of the most basal parts of this region. The cell groups which originate from this matrix eventually are located basal and lateral to the ones just described. These cell groups are: the suprapeduncular complex and the corpus subthalamicum Luysi. The cellular elements which are going to form these nuclei migrate from the matrix in a lateral direction; the stream of migrating cells curves around the earlier differentiating structures and becomes located in a position superficial to the latter. Within the suprapeduncular complex a pars geniculata and a pars incerta were discerned. The pars geniculata is located at the level of the zona limitans intrathalamica and probably gives origin to the ventral parts of the geniculate bodies. The pars incerta constitutes the caudal part of the eventual zona incerta. The corpus subthalamicum Luysi, another genuine constituent of the regio subthalamica, originates from the recessus supramamillaris, caudal to the mamillary primordium. Hence, its cells shift superficially into a rostrodorsal position. In earlier stages we observed an early differentiating zone located just basal to the sulcus diencephalicus ventralis. This zone which extends from the regio subthalamica rostrally to the foramen of Monro we called the subthalamic cell cord and it is located in the same position as the longitudinal subthalamic zone described by Kahle ('56) and Richter ('65) in a human material. According to the latter author this cell cord gives origin to globus pallidus, nucleus entopeduncularis and corpus subthalamicum Luysi. As far as the corpus subthalamicum Luysi is concerned, we concluded that it took its origin from the matrix of the recessus supramamillaris.

The diencephalic origin of the nucleus entopeduncularis and the globus pallidus as suggested by Spatz ('21, '24, '25), Kuhlenbeck ('24, '27, '54), Kahle ('56) and Richter ('65), is further substantiated by the present study. In our opinion, however, it remains to be proven from which matrix area these nuclei develop. Judging from the material studied, the possibility could not be discarded that in early stages a continuity exists between the regio subthalamica and the basal telodiencephalic border. The regio subthalamica does not reach beyond the rostral border of the parencephalon anterius as far as its ventricular matrix is concerned, but by way of migratory translocations its cells extend laterally in the direction of the basal telodiencephalic boundary region, in the meantime pushing apart the thalamic and hypothalamic structures.

Hypothalamus

The hypothalamus of the official terminology comprises all structures located in the diencephalic wall basal to the sulcus diencephalicus ventralis, and most authors consider this region as a single ontogenetic unit (see for example His, 1888, 1893[a]; Herrick, '10,'17; Kuhlenbeck, '29, '30). However, in our embryological approach of this part of the brain, we were struck by the heterogeneity of the constituent parts of the hypothalamus. Therefore, we judged it preferable to discuss these different regions separately. Four regions could be distinguished (*i.e.*, the regio mamillaris, the regio postoptica, the regio supraoptica and the regio praeoptica) which appeared as four separate ontogenetic units.

The mamillary region is located rostrobasal to the subthalamic region.

The postoptic region is situated rostral to the former and comprises what is commonly called the infundibulum. The hypothalamic cell cord (Gilbert, '35) is the dorsalmost constituent of this postoptic region and extends from the subthalamic region to the optic chiasm. Early differentiating nuclei (nucleus ventromedialis) as much as nuclei that develop in a rather protracted way (nucleus infundibularis) are present.

The supraoptic region can be discerned dorsorostral to the postoptic region, and it shows a remarkable increase in volume during development. It contains among others the paraventricular and supraoptic nuclei. Its rostral margin is marked at the ventricle by the presence of the sulcus intraencephalicus anterior.

The preoptic region is situated rostral to this sulcus and forms the rostralmost division of the hypothalamus.

During early development the four divisions just mentioned show considerable independence; later on the medial forebrain bundle, which passes through the lateral parts of these regions, tends to give the hypothalamus a rather uniform appearance. The corpus mamillare, however, retains its independent position and even in the adult brain remains characterized by features distinguishing it from the rest of the hypothalamus (as for instance its wealth of myelinated fibres).

CONCLUSION

From this discussion it appears that the so-called horizontal or longitudinal cell columns of the adult diencephalon actually develop within the embryonic mantle layer as constituents of originally transverse parts of the neural tube. These transversely oriented parts are the neuromeres which express the existence of proliferation maxima within the wall of the early neural tube. Within these neuromeres, migration areas reveal themselves as development proceeds, and these areas form the ontogenetic units that together constitute the adult brain.

A number of factors obscures the original relationships during later stages of development, as for example the various modes of cell migration within the mantle layer and the development of fibre tracts which push the grisea apart. The transformations of the neural tube, caused by the differential growth and by the differentiation of the areas, is another factor which tends to obscure the microscopist's view of the whole of the diencephalon and of its internal relationships. In the embryological part of our study it appeared that the adult configuration as represented by the His-Herrick-Kuhlenbeck scheme manifests itself only gradually. It is preceded by stages in which more fundamental units develop. These have to be considered the primary ontogenetic units which together constitute the diencephalon. The relationships between these primary units and the secondary longitudinal columns can be formulated readily, provided that a sufficient number of stages is studied between the early neuromeric brain tube and the adult configuration of the brain. Thus, the actual findings of those authors who studied the mammalian diencephalon along the His-Herrick-Kuhlenbeck doctrine are not subject to criticism as far as the naming and the recognition of the nuclei is concerned; we are very reluctant, however, to accept the interpretation of the so-called horizontal or longitudinal zones as fundamental units in a comparative sense and the results of our study of the development of the diencephalon in a mammalian embryo do not sustain this hypothesis.

In our opinion the adult configuration, being the final result of a gradual self-subdivision of the brain, cannot form the sole basis to support the building of comparative neuroanatomy. We suggest an embryological approach which starts early enough in development to recognize the, in our opinion, fundamental neuromeric pattern.

CHAPTER X

SUMMARY

In this thesis the development of the diencephalon of the Chinese hamster has been analysed. The different criteria of subdivision of the diencephalon, *i.e.*, neuromerism (Bergquist, '32, '52; Bergquist and Källén, '54), ventricular relief (His, 1893[a]; Herrick, '10; Kuhlenbeck, '30, '54), matrix activity (Kahle, '51, '56) and mantle layer differentiation (*i.e.*, the development of grisea) have been tested on their validity and mutual consistency by studying the development of one single species.

The Chinese hamster was chosen as a test object because of the accuracy that could be attained in the determination of the postcoital age of the embryos. Gestation in this animal lasts twenty-one days. Our study focusses on the developmental period extending from the moment of closure of the anterior neuropore (*i.e.*, $E_{10^1/4}$) to the adult stage (*i.e.*, PN_{100}).

In the study of the midline configuration (cf. fig. 4) it was demonstrated that the shape of the neural tube is subject to strong transformations during successive stages of ontogenesis and it appeared that knowledge of these transformations is of paramount importance for the interpretation of the microscopical picture. The transformations of the midline structures in the wall of the third ventricle are such that the various landmarks (*e.g.*, epiphysis, commissura posterior, infundibulum and chiasma opticum), although they retain the same topological position, show considerable topographical translocations. We compared these combined movements to the movement of a transport band at its turning point. These movements also affected the course of the central brain axis. Therefore, the interpretation of serially sectioned embryos becomes difficult as the true transverse plane changes according to the part of the brain under consideration. For this reason we decided to make frequent use of graphical and three-dimensional reconstruction techniques.

Neuromeric phenomena could already be observed in the neural plate stage (E_{10}). Neuromerism reaches its climax at the E_{13}-stage. At this stage six neuromeres can be discerned within the prosencephalon, *i.e.*, a telencephalic neuromere, an optic neuromere, a postoptic neuromere, a parencephalon anterius, a parencephalon posterius and a synencephalon (cf. figs. 15 and 16b).

The diencephalic borders at this stage can be recognized clearly and consist of ventricular eminences corresponding to external constrictions. The telodiencephalic border, which at this stage corresponds to the border between telence-

phalic and optic neuromere, later on becomes transformed beyond recognition. It appeared that the generally accepted telodiencephalic border formed by structures surrounding the foramen of Monro most probably does not correspond to this early embryonic border. The syn-mesencephalic border is also subject to profound changes and at later stages no single transverse plane can be indicated to form the boundary-plane between diencephalon and mesencephalon.

The ventricular surface of the diencephalon was studied at older stages and a longitudinal sulcus, *i.e.*, the sulcus diencephalicus ventralis s. hypothalamicus, was observed. A number of authors consider this sulcus to be the rostral continuation of the sulcus limitans of His. The sulcus limitans is reported to mark the boundary between the sensory alar plate and the motor basal plate This sulcus has been traced into the diencephalon by several authors with different results. According to Kingsbury ('30) and Kuhlenbeck ('48) the sulcus limitans ends in the recessus mamillaris; His (1893[b]), Johnston ('09) and Streeter ('11) were of the opinion that it ends in the preoptic recess, and Spatz ('27), Grünthal ('52), Kahle ('56) and Richter ('65) thought that it ends in the foramen of Monro. Our analysis revealed that within the synencephalic region a wide depression exists through which all kinds of continuities between mesencephalic and diencephalic sulci can be postulated. Furthermore, the pattern of the sulci is changing continuously. Therefore, the isolated study of different stages can lead to totally diverging interpretations. Our observations revealed that the concept according to which a rostral continuation of the sulcus limitans serves as an important morphological landmark in the diencephalon, is untenable.

As regards the remaining sulci in the diencephalon it appeared that they can be subsumed under two categories. A number of them can be related directly to remainders of the neuromeric bulgings and as such they can be characterized as originating transversely; the recessus preopticus and part of the sulcus intraencephalicus anterior can be considered a remnant of the optic neuromere; the sulcus lateralis infundibuli is a derivative of the parencephalon anterius; part of the sulcus diencephalicus dorsalis may be considered a relic of the parencephalon posterius cavity; the synencephalic recess (or: recessus metathalamicus) represents the synencephalic cavity (cf. fig. 32). The second category of sulci forms a heterogeneous group, originating as secondary effects of differences in development of parts of the wall of the neural tube, which are not related directly to neuromerism.

The study of the sulci taught us, in addition, that their mutual configuration is subject to change during development and that they can not be used as landmarks in the study of the development of structures in the wall of the neural tube of the Chinese hamster.

The histological development of the wall of the diencephalon was studied in the light of the recently developed ideas about the dynamics of histogenesis of

the brain (Sauer, '35a, b; Sauer and Walker, '59; Fujita, '60, '62, '63; Berry and Rogers, '65). The pattern of heterochrony as defined by Spatz ('27) and Kahle ('51, '56) has been thoroughly analysed. To this end the histogenetic process was subdivided into 9 phases and with the help of a code the developmental state of the diencephalic matrix was mapped out in graphical reconstructions (cf. figs. 33a and 33b).

As far as heterochrony is concerned a number of salient features could be observed within the diencephalon; a) a general caudorostral gradient was seen in the rate of development of the matrix; b) the interneuromeric borders were progressive when compared to the adjacent matrix areas; c) within the caudal diencephalic neuromeres a general basodorsal gradient was observed (cf. fig. 18).

The prerubral tegmentum is the earliest area within the diencephalon in which matrix development gives rise to differentiation. From this progressive area, differentiation spreads in both a rostral and a dorsal direction. The hypothalamic cell cord and the subthalamic cell cord, which are more or less horizontally oriented cell columns, develop as rostral extensions of this prerubral tegmentum. Two regions within the basis of the diencephalon, however, are characterized by a considerably retarded maturation of the matrix, *i.e.*, the part of the hypothalamus basal to the hypothalamic cell cord, situated between the mamillary anlage and the optic chiasm, and the basalmost part of the preoptic region.

As regards the dorsal part of the diencephalon, acceleration of interneuromeric crests and retardation of neuromeric bulges are observed in the synencephalon, parencephalon posterius and parencephalon anterius. Within the interneuromeric areas fibre streams are seen developing between the early differentiating neuroblasts. In this way some interneuromeric borders can be followed from the early embryonic stages up to the adult stage. The syn-mesencephalic border is characterized by the presence of the posterior commissure and the syn-parencephalic border by the fasciculus retroflexus. In the interparencephalic border a fibre lamina develops, *i.e.*, the lamina medullaris externa, which in earlier stages is preceded by the tract of the zona limitans. These interneuromeric borders thus form well-defined landmarks that can be used in the study of the wall of the neural tube.

In general, a basodorsal gradient of matrix development was also observed in the neuromeric, transversely oriented, parts of the diencephalon. The pars dorsalis thalami is an exception to this rule and, compared to its surrounding structures, remains strongly retarded during a considerable period of development. When differentiation of the pars dorsalis thalami has started, however, its development is disproportionate and soon the pars dorsalis thalami is seen pushing aside the neighbouring areas.

The eventual configuration of the mantle layer is only indirectly related to matrix activity. This follows from the mere fact that sooner or later the process of matrix development takes place in all regions of the diencephalon. The pat-

tern of matrix development, however, as shown by the matrix phase maps, is clearly mirrored by the sequence of appearance of mantle layer structures; knowledge of this pattern adds to the understanding of the construction of the diencephalic part of the brain. The mantle layer structures which could be recognized were reconstructed (cf. figs. 51, 54 and 57).

Various modes of migration occur in the process of mantle layer formation. The most common way of migration is a radial one by which differentiated cells are deposited external to the matrix area from which they originate. A tangential migration was observed in the development of the corpus subthalamicum Luysi, the suprapeduncular complex and part of the substantia nigra (cf. fig. 58); the inverted-fountainhead-fashion in which the cells of these structures migrate might be a characteristic of the structures derived from the original, primarily continuous part of the neural plate extending between the fovea isthmi and the optic chiasm.

The conclusions about the migration path in the diencephalon were partly based on autoradiographic material. The autoradiographic technique proved to be a valuable tool in the study of diencephalic development: it adds the factor time to the otherways static histological picture.

The units which have developed from the neuromeres can be recognized throughout development. In the adult diencephalon this pattern is obvious especially in the caudal parts. Here the pretectal region with its commissural and precommissural parts represents the dorsal half of the synencephalon. The nucleus posterior thalami is also a derivative of the synencephalon and the synparencephalic border passes rostral to this nucleus. The border itself is marked by the presence of a fasciculus retroflexus. The epithalamus and the pars dorsalis thalami are both derivatives of the dorsal half of the parencephalon posterius. Rostral to the latter the lamina medullaris externa develops at the place of the interparencephalic border. The basal representatives of the synencephalon and the parencephalon posterius do not show any clear boundaries: here we consider the prerubral tegmentum a derivative of the basal synencephalon; the basal representative of the parencephalon posterius is formed by the regio subthalamica and gives origin to the suprapeduncular complex and the corpus subthalamicum Luysi.

Rostral to the interparencephalic border three neuromeres were discerned at early stages, *i.e.*, the parencephalon anterius, the postoptic neuromere and the optic neuromere. Here the interneuromeric borders fade because of the early development of differentiated cell columns (the subthalamic and hypothalamic cell cords). Therefore, no neuromere-derived subdivision of this part of the diencephalon could be detected in the adult brain. A number of ontogenetic units, however, could be distinguished. The dorsal part of the parencephalon anterius remains recognizable and develops into the pars ventralis thalami. A separate subthalamic cell cord is seen dorsal to the hypothalamus of later stages.

Within the hypothalamus four ontogenetic units are discerned, *i.e.*, a regio mamillaris, a regio postoptica, a regio supraoptica and a regio preoptica (cf. fig. 55).

In this study on the development of the diencephalon of the Chinese hamster it appeared that during early phases of development ontogenetic units can be discerned which are in part characterized by direct relationships with neuromeres. Knowledge of these ontogenetic units and of their relationships is of great importance for the establishment of homologies.

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STELLINGEN

I

De grondige kennis van de morfologie van het zenuwstelsel blijft ook in de huidige tijd met zijn geavanceerde biochemische, neuroradiologische en neurofysiologische technieken het hechte fundament waarop de klinische neurologie is gebouwd.

II

De opvatting dat de beide mediale schorsvelden van reptielen homoloog zouden zijn met de ammonshoorn en de fascia dentata van zoogdieren is onbewezen.

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III

Bij de behandeling van acute lymfoblasten leukaemie verdient het aanbeveling een therapie in te stellen gericht op het voorkómen van meningeale en cerebrale complicaties.

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IV

Bij de behandeling van jeugdige epileptici met anticonvulsiva dient men bedacht te zijn op de ontwikkeling van een vitamine-D deficientie.

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V

1,25-dihydroxycholecalciferol wordt door de nier gevormd uit een metaboliet van vitamine D en naar het bloed uitgescheiden Deze stof kan worden beschouwd als een hormoon dat de calcium resorptie uit de darm reguleert.

D E M Lawson et al (1971) Nature (Lond), 230, 228 230

VI

Tussen de infantiele spinale spieratrofie van Werdnig-Hoffmann en de juveniele spinale spieratrofie van Wohlfahrt-Kugelberg-Welander is er plaats voor een derde vorm van spinale spieratrofie.

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VII

De diagnose Morbus Batten (de zogenaamde neuronale ceroïd lipofuscinose) en de differentiatie tussen zijn laat-infantiele en juveniele vorm kan gesteund worden door de electronenmicroscopische bevindingen in een perifeer zenuwbiopt.

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VIII

Tussen de ziektebeelden 'essentiele (hereditaire) tremor' en 'essentiële (hereditaire) myoclonie' bestaan overgangsvormen. Het vóórkomen ervan in één familie suggereert dat beide ziektebeelden verschillende uitingsvormen zijn van één genotype.

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IX

Het vóórkomen bij de carotisangiografie van het 'tram-track fenomeen' wijst in feite slechts op een laesie van de arteria meningea media en de erbij horende vene en niet zonder meer op een epiduraal haematoom.

X

Op grond van het verloop van de stam van de arteria cerebri media en haar insulaire takken in het voorachterwaartse carotisangiogram is het mogelijk een temporaal gelocaliseerde contusie of een temporaal gelocaliseerd intracerebraal haematoom te differentieren van een onder de slaapkwab gelegen epiduraal haematoom.

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XI

De motorische geremdheid van een lijder aan een vitale depressie en de hypokinesie van een aan paralysis agitans lijdende patiënt zijn twee uitdrukkingsvormen van één gemeenschappelijke biochemische grondstoornis.

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H. M. van Praag en J. Korf (1971): Ned. T. Geneesk., 115, 514-515.

XII

Het zou zowel de financiele positie van de universiteit als de kwaliteit van het onderzoek ten goede komen, indien onderzoekers, alvorens een gepland researchproject te mogen verwerkelijken, een periode van bezinning zouden moeten doormaken.

Nijmegen, 15 december 1972 A. KEYSER